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STUDIES OF ACARI

SECOND SERIES: DESCRIPTIONS OF NEW SPECIES AND NOTES ON ESTABLISHED FORMS OF PARASITIC MITES

BY FRANK A. TURK, F.R.E.S., F.Z.S.

(With 19 Figures in the Text)

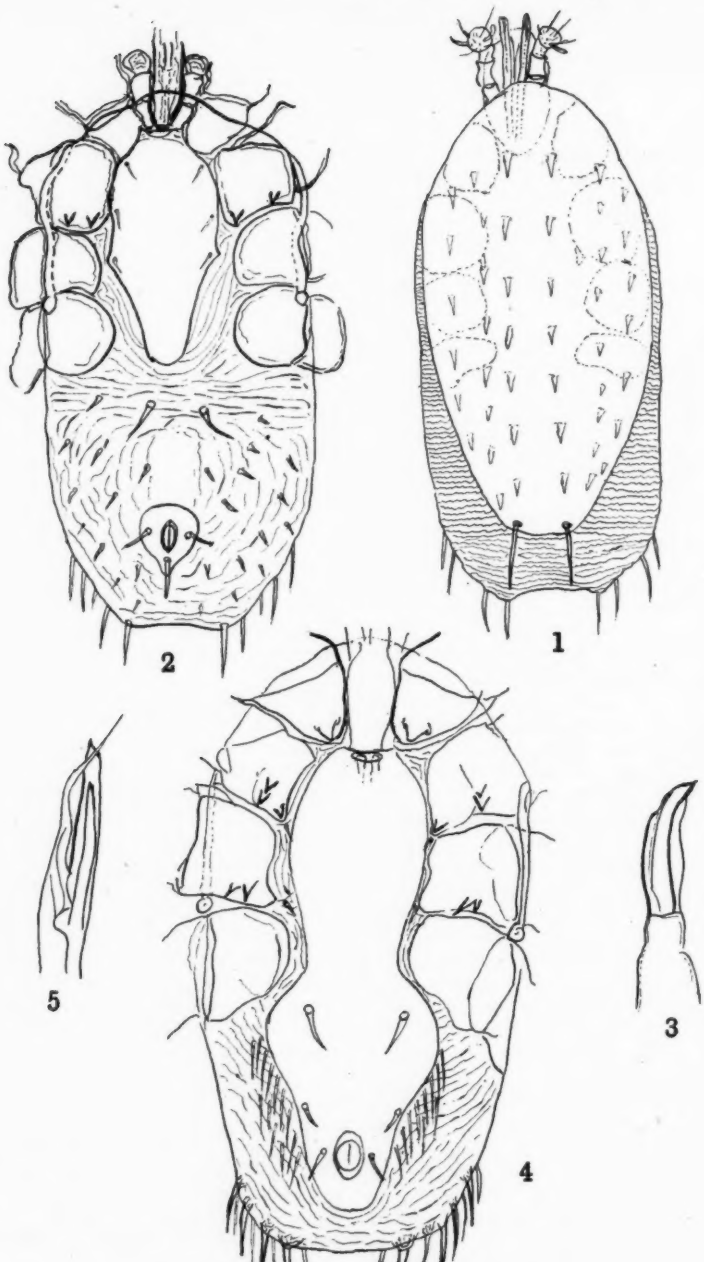
The present paper deals with descriptions of some new species of parasitic mites, a fresh study of the forms of *Eulaelaps stabularis* (Koch), some observations on the occurrence of hypopial forms on other parasitic arthropods, and finally some observations on Radford's 'Genera and species of parasitic mites' (1943).

ICHORONYSSUS SORICIS N.SP.

This species is closely allied to *Ichoronyssus albatus* (Koch), but is certainly distinct in both the stages known to me. It is probable that there is a species complex, the forms of which are all in near relationship to *I. albatus*. Oudemans (1902) described one such form under the name of *I. albato-affinis* Oudemans (a species not mentioned in Radford's list—see my remarks later in the present paper). The only forms I possess at present of this new species are the nymph and the male.

Nymph. Dimensions: 352 μ long, 165 μ broad. Colour, in life, is a very pale yellow. The dorsal shield (Fig. 1) leaves a large amount of the dorsum uncovered, the skin of which is quite strongly and finely horizontally striated. The shield terminates at a greater distance from the posterior border of the body than in *I. albatus* (Koch) and the shield is more truncate posteriorly than in that species. On the dorsal shield are six rows of spine-like hairs shorter and stouter than in *I. albatus*. On the posterior border of the shield is the usual pair of long terminal hairs. Ventrally there is a long sternal shield (Fig. 2) and a small anal shield. In all my three specimens I have been able to make out only three pairs of hairs on the sternal shield. Oudemans (1902) says that in *I. albatus* 'the sternal shield is long with four pairs of hairs'. The peritreme reaches only to the anterior border of the second coxae. In *I. albatus* it attains a slightly more forward position. The first and second pairs of legs are thicker than the third and fourth and the spines on them appear to be situated as follows: on the coxae of the second pair of legs two posterior spines; on the femur of the second pair one dorsal spine; on the coxae of the third pair one small (vestigial?) posterior spine. This spinulation of the legs shows a reduction on that found in *I. albatus*. Between the sternal and anal shields is a pair of stouter and longer spines. The uncovered skin of the ventral surface is not so strongly striated as that of the dorsal surface nor are all the striations so markedly horizontal. The posterior border of the body is slighly concave in all my specimens. The mandibles are toothless, very stout and slightly sinuate before the apex.

Male. Dimensions: 555 μ long, 270 μ broad. Colour darker and browner than that of the nymph. Dorsally this species is very similar to *I. albatus* and the males of other species of this genus. On the ventral surface the following features are diagnostic of this species: the sternal area of the sterni-geniti-ventri-anal shield is entirely devoid of hairs, but detailed examination has revealed two pairs of very minute hairs on the unchitinized skin between the shield and the coxae, one at the interior posterior angle of the second coxae and the other similarly placed by the third coxae (Fig. 4). On the ventri-anal portion of the shield are three pairs of longer and more robust hairs. Of these the anterior pair is the longest. On the posterior border of the first pair of coxae is a single stout blunt spine together with one dorsal spine on the femur of the first pair of legs, although this latter is small, and, on the type specimen, difficult to make out. On the posterior border of the coxae of the second pair of legs are three spines and on the coxae of the third pair of legs two spines, all of which are placed as shown in Fig. 4. There is also one dorsal spine on the femur of the second pair of legs. The arrangement and disposition of the other hairs of the ventral surface are shown in Fig. 4 and are better figured than described. These show slight differences from the condition found in *I. albatus*. The mandible is very different from that of *I. albatus* as figured and described by Oudemans (1902). The movable ramus is stout and obliquely truncate without any teeth. The fixed ramus is, distally, drawn out into a long, extremely slender spine and in the proximal



Figs. 1–5. *Ichoronyssus soricis* n.sp.

Fig. 1. Nymph, dorsal.
Fig. 2. Nymph, ventral.
Fig. 3. Nymph, mandible.
Fig. 4. Male, ventral.
Fig. 5. Male, mandible.

third is a strong tooth (Fig. 5). I have never met this form of mandible in the males of any other species of *Ichoronyssus*. Three nymphs and one male taken from *Sorex minutus* L. captured on the North Cliffs near Gwithian, Cornwall, 16 May 1942.

Types, all on a single slide, in my collection.

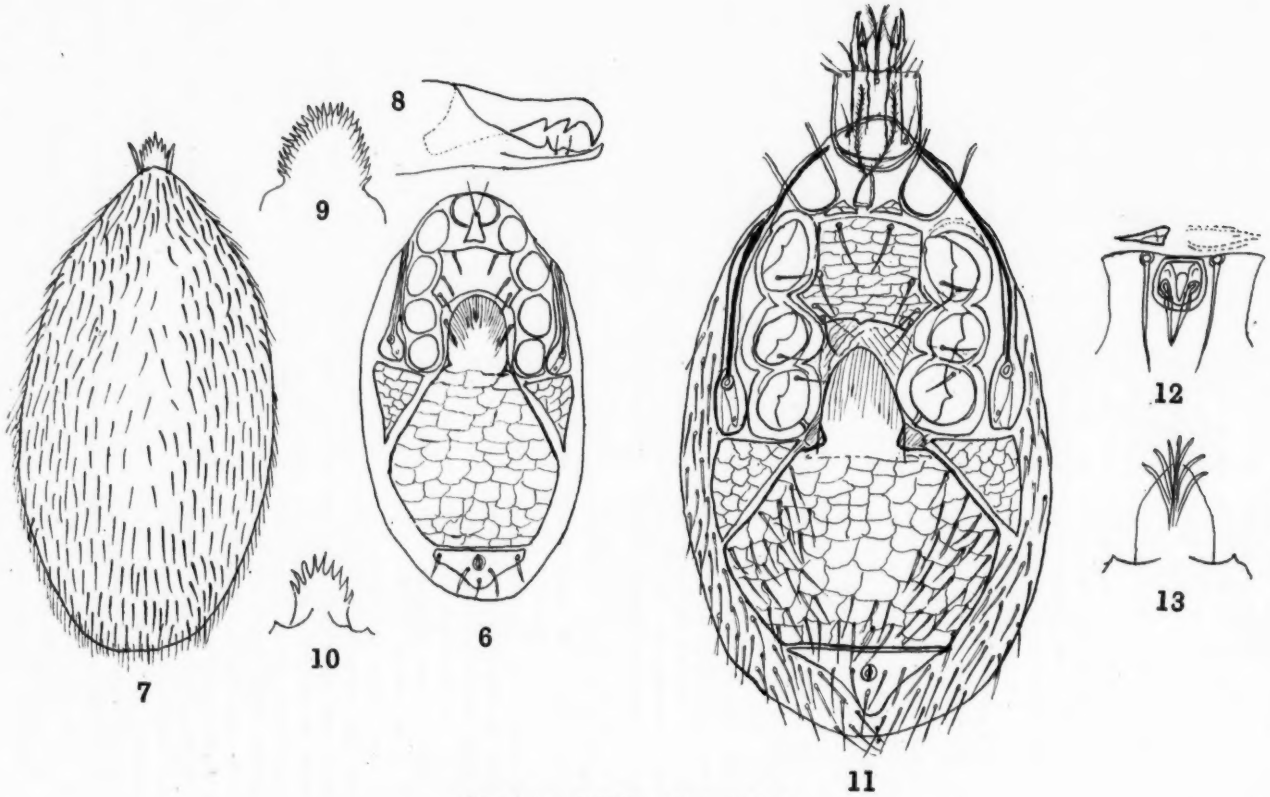
EULAELAPS STABULARIS (KOCH)

This is by far the commonest species of acari to be found in the nests and on the bodies of small rodents and insectivores. Koch (1836) first described the species and it was later referred to his genus *Laelaps* Koch (1842). In 1903 Berlese instituted the genus *Eulaelaps* for this species, which was made the type of the new genus. In 1913 Oudemans described and figured what he believed to be all the stages of this mite under the name of *Hypoaspis stabularis* Koch. In the course of this work Oudemans mentions that all the females which he found in the nest material were dark brown, whilst those taken on moles caught in a trap were much lighter in colour. It was this last observation which has prompted me to make a fresh study of all my material of this species.

Structural differences between these light and dark individuals were soon apparent. These may be best seen, in the case of the females, by comparing Fig. 6 (one of the dark specimens) with Fig. 11 (one of the lighter specimens). The major differences may briefly be set forth as follows: the smaller and darker forms have no jugularia; three pairs of hairs are present on the sternal shield; the anal shield possesses a hair at each of the anterior lateral angles; the posterior expansion of the peritrematal shields is subcircular. In the larger, lighter coloured individuals jugularia are present; the anterior lateral angles of the anal shield are without hairs; on the sternal shield four pairs of hairs are present and the posterior border of the sternal shield is much straighter than in the darker specimens; the posterior expansion of the peritrematal shields is much more elongate and somewhat truncate posteriorly. The differences between the two forms of the male are not so marked, but a comparison of my Fig. 7 with Oudemans (1913) figure *A* of a male shows that my specimen has a more elongate body which is also more truncate posteriorly. Males of the darker, smaller type are without the jugularia present in the lighter individuals. Further differences between these two types are to be found in the epistomes. Fig. 9 represents the epistome of a small dark type female and Fig. 10 that of a similar male, whilst Fig. 13 is the very lightly chitinized epistome of one of the larger lighter males. Males of this latter type also have a more complexly chitinized penis which is shown in Fig. 12. The same figure, which was drawn from a specimen taken from *Apodemus sylvaticus sylvaticus* (L.), shows one of the jugularia rudimentary and one fully chitinized.

That two distinct forms have hitherto been indiscriminately recorded under the same specific name now seems certain. The question now remains whether these two forms are two distinct species. In attempting to answer this question the knowledge of some further facts is of importance. The females of both types may be found containing fully developed eggs which, as far as my observations go, are laid singly. In the examination of a great number of specimens I have never taken the larger lighter individuals from nest material, but they always occurred on the hosts. The smaller darker type is to be found in the greatest numbers in the nest material, but they also occur frequently on the hosts. I have records of both types from the nests of, or as ectoparasites upon, the following hosts: *Talpa europaea* L., *Sorex araneus castaneus* Jen., *Erinaceus europaeus* L., *Apodemus sylvaticus sylvaticus* (L.), *Rattus norvegicus* (Erxl.), *Mus musculus* L., *Sciurus vulgaris leucourus* Kerr (light and dark specimens on the host—no records from nest material), *Sciurus carolinensis* Gmel. (dark individuals from nest material only). Thus both forms are distributed over a wide range of hosts and the habits of both forms and their occurrence in the nest material or on the host animal respectively seem to be the same for every host species. In addition to this, the facts set out above show that the lighter forms found only on the bodies of the mammals they parasitize represent an advance in structure, and in the specimen in Fig. 12 one of the jugularia has actually been observed in process of formation. In view of all these facts I am of the opinion that the small dark individuals are the tritonymphs and the lighter specimens are the adults of the same species. We have here an example of Neoteny such as is most familiar in certain kinds of Amphibia. Only when the tritonymphs adopt a truly parasitic habit do they continue their development to the adult stage. It seems possible, however, that they may breed and continue a free-living or pseudoparasitic existence in the nest material and perhaps on the host for several generations.

It has been pointed out above that the male tritonymph commonly occurring on the different hosts in this country and figured in Fig. 7 differs slightly from that figured and described by Oudemans (1913). There are differences also to be found in the female tritonymph. The most obvious difference is to be seen in the anal shields; Oudemans (1913) represents this as not being continuously contiguous with the posterior border of the ventral shield, whilst in all the examples I have examined and figured it is so. In Fig. 8 I have drawn the mandible (chela) of a female tritonymph from the



Figs. 6–13. *Eulaelaps stabularis* Koch.

Fig. 6. Female tritonymph, ventral.
Fig. 7. Male tritonymph, dorsal.
Fig. 8. Mandible of female tritonymph.
Fig. 9. Epistome of female tritonymph.
Fig. 10. Epistome of male tritonymph.
Fig. 11. Adult female, ventral.
Fig. 12. Penis and jugularia of adult male.
Fig. 13. Epistome of adult male.

(*Note.* No body hairs are shown in Fig. 6 for the sake of clearness.)

nest of a Grey Squirrel. The chief difference between this and that illustrated in Oudeman's paper is that the English specimens have two hairs on the fixed ramus of the mandible which also has a more procumbent apical tooth. I was much puzzled by these differences in view of the fact that the descriptions of the protonymphs and deutonymphs of *Eulaelaps stabularis* given by Oudemans agree perfectly with those with which I am acquainted. I sent a note concerning this, together with a tracing of Oudeman's figure of the female and my own drawings, to Dr J. E. Hull, D.Sc., of Belford, Northumberland, who, besides being the leading English authority on the Acari, has also had much correspondence with the late Prof. Berlese. In the course of his letter, sent in reply, he states that none of his specimens agrees with the figure and description given by Oudemans and he says further (*in litt.*) 'this is certainly not the *Eulaelaps stabularis* (Koch) as understood by Berlese'. He has also suggested that the appearance of these structures in Oudeman's drawings may possibly be due to the collapse of the chitinous parts when mounting. Reference to Koch's original descriptions has confirmed me in the belief that the specimens under examination by Berlese, Hull and myself are in fact referable to Koch's species and are the true *E. stabularis* (Koch). It has been impossible to correspond with Dr Oudemans during the present war and the death of Graf H. Vitzhum in May of last year (1943) has prohibited my gaining the opinion of the only other authority in a position to throw some light on the matter. Under these circumstances it appears best to propose a new name for Oudemans' form which I suggest should be *Eulaelaps oudemansi* n.nom. = *Hypoaspis stabularis* Ouds. 1913 (non Koch 1836) tritonymph only.

Oudemans (1913) has suggested that *Laelaps oribatoides* Michael (1892) is a synonym of *Eulaelaps stabularis* Koch. I have, however, two specimens, both females, taken from a mole's nest at Reskadinnick, Camborne, Cornwall (the county from which Michael obtained most of his material) which agree very well with his description in shape, size, structure of mandibles and epistome as well as in having the whole of the ventral surface covered with hairs as described by Michael (1892). In this last respect, as in the structure of the mandibles and to a lesser extent the epistome, they would appear to be distinct from *E. stabularis* (Koch) although certainly very closely allied. Attention might be drawn to the fact too that the dimensions of the male as given by Michael are very much less than those met with in *E. stabularis*. I am therefore of the opinion that *E. oribatoides* (Michael, 1892) should be retained as a good species, at least until the males can be re-examined.

Fig. 9. Epistome of female tritonymph.

Fig. 13. Epistome of adult male.

(*Note.* No body hairs are shown in Fig. 6 for the sake of clearness.)

MYOBIA PHILLIPSI N.SP.

I possess only the male of this very distinct species which is figured in Fig. 14. The fact that it differs from all other species of the genus in the extraordinary length of the dorsal hairs warrants the present description and the institution of the species. Dimensions: 200 μ long, posterior hairs 265 μ. The body is shorter and broader than most other forms of the genus and is perhaps most nearly like that of *Myobia ingens* Vitzhum (1914). The usual pair of posterior dorsal hairs is one and a third times the length of the body and set on conspicuous tubercles. Between the first and second

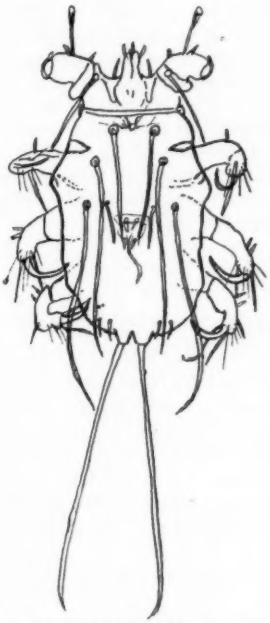


Fig. 14. *Myobia phillipsi* n.sp.

Male, dorsal.

pairs of legs is a median pair of hairs reaching to the penis which, as is usual, opens dorsally and has two pairs of very short delicate spines set round it, which are very difficult to see. Other dorsal hairs are adequately shown in the figure and are quite sufficient to distinguish this species from any other of the genus. The claws to all the legs are outstandingly large and well chitinized. The tarsi of the last three pairs of legs are furnished with several long spine-like robust hairs, each nearly as long as the tarsal claw. On the pre-femur of the first legs is a long, backwardly directed spine reaching to the second legs and upon the femur of the first legs is an anteriorly projecting spine-like, capitate hair,

easily seen in the type and very distinctive. The single specimen for which the species is instituted was found by Miss S. M. Phillips on a water shrew *Neomys fodiens bicolor* (Shaw), and I have pleasure in naming this most interesting and distinctive form after its discoverer. The host was captured in Green Lane, Truro, Cornwall, on 11 April 1943. It is possibly an uncommon species, as this was the only specimen taken after careful search and other specimens of the host have failed to furnish any further material. In my experience the host is a mammal peculiarly free from ectoparasites of any kind. The type is in my own collection.

It may be remarked here that all the forms of *Myobia* which are parasitic on shrews agree in having little or no proximal expansion to the dorsal spines and in this respect are the antithesis of the species found infesting bats. It seems that the time is now ripe for some acarologist to work over the species comprised in this now somewhat unwieldy genus with a view to seeking possible relationships between forms parasitizing related hosts much as has been done by entomologists with the Mallophaga comprised in the old genus *Philopterus* Nitzsch.

LEPTUS (*ACHOROLOPHUS*) *KILLINGTONI* N.SP.

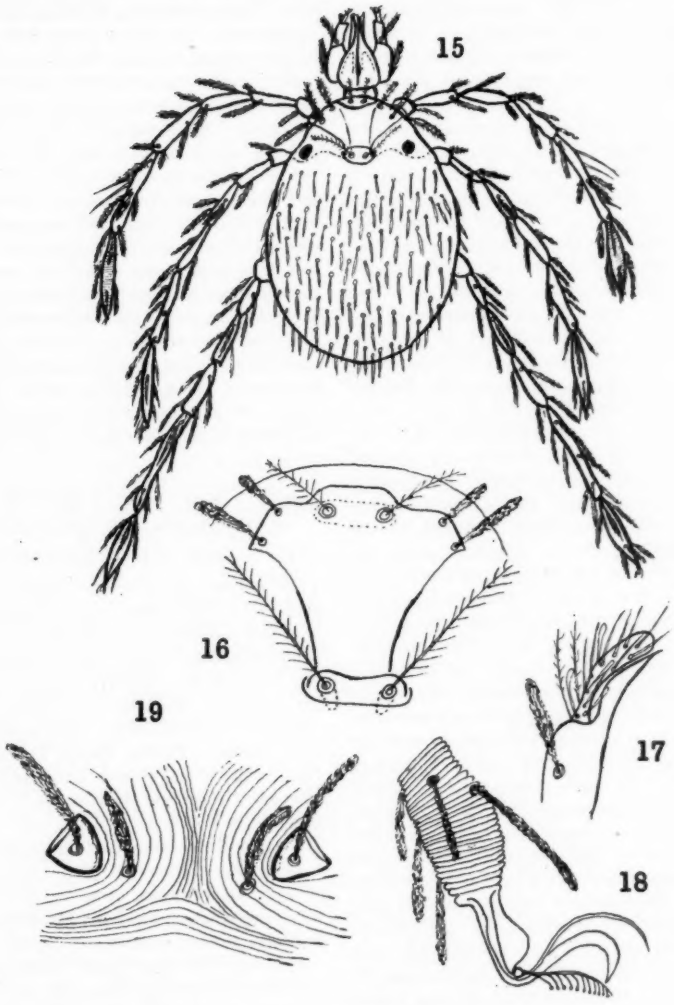
On 6 September 1943 Dr F. J. Killington sent to me for identification a *Leptus* larva which he had taken frequently as a parasite of dragonflies. He also informed me that Münchberg (1935) had recorded a form of *Leptus*, which he referred to *L. ignotus* Ouds. (1903) as a parasite of dragonflies. The species found by Dr Killington proves to be new to science and I have to thank him for this opportunity to describe it and have pleasure in naming it after its discoverer.

The general form of the mite is shown in Fig. 14. In many respects it resembles *L. ignotus* (Ouds.), but the body is far more ovoid. The hind three-fourths of the dorsum is covered with small rod-like hairs a little more profuse than is generally found in the species most nearly related to it. The dorsal shield is shown in Fig. 16 bearing the usual two pairs of pseudostigmatic hairs; the other hairs of the shield are plumed to the base as in the majority of the forms of the genus. The anterior part of the shield itself in the specimens sent to me is very poorly chitinized, but as far as I can distinguish the anterior border is prolonged into a forward projecting convexity. The palpal claw, Fig. 17, is broadest about the middle, sharply pointed and much recurved. The palpal organ has several types of sensory hairs: (*a*) the two sparsely plumose ones at the proximal end which are long and easily seen and possibly a useful diagnostic character, (*b*) a few clavate hairs rather rod-like, and (*c*) a large number of moderately long, sharply pointed hairs. The tarsi of the first pair of legs are ringed and this ringing, being quite distinct, serves as a useful distinguishing feature in connexion with the general shape of the body and the character next to be described. On each tibia of the first pair of legs are two fine, pointed, unplumed sense hairs situated about the middle of the segment: these do not appear to occur on any of the other legs of the specimens which I have examined. Fig. 18 shows the tarsus of leg 1 from the underside. The relationships of the claws and the pulvillus are sufficiently obvious from the figure. Between the coxae of the first legs is a pair of hairs shorter than those situated on the coxae and themselves placed nearer to the coxae than in the specimens of any other species which I have examined. The arrangement of these hairs is shown in Fig. 19.

From particulars sent to me by Dr Killington I quote the species of Odonata on which this parasite has been found: *Pyrrhosoma nymphula* (Sul.), *Enallagma cyathigerum* (Charp.), *Coenagrion puella* (Linn.), *Palaeobasis tentella* (Vill.), *Lestes sponsa* (Hans.), *Cordulegaster boltonii* (Donovan), *Anax imperator* Leach, *Orthetrum coerulescens* (Fab.). All the specimens so far known were taken from three habitats on Alderney Heath on the northern outskirts of Parkstone, Dorset. The only common feature of the three habitats is the tall rank vegetation. Dr Killington says (*in litt.* 13 November 1943): 'The dragonflies are attacked mainly after they have reached a mature stage. Parasites attach themselves to all parts of the insects, except that none has been found on the wings. The legs are the usual place for attachment. The greatest number of parasites found on one dragonfly was eight. The first dragonfly taken with *Leptus* was on the 12th June 1943 and the last on 11th August 1943. Altogether one hundred and eighteen dragonflies were taken with this mite.'

Dimensions: Length, 610 μ with rostrum; breadth 390 μ; length of first legs 620 μ.

The type, in accordance with Dr Killington's wishes will be placed in the British Museum. Cotypes in the Oxford University Museum, Dr Killington's collection and my own collection.



Figs. 15–19. *Leptus killingtoni* n.sp.

Fig. 15. Larva, dorsal.
Fig. 16. Dorsal shield of larva.
Fig. 17. Palp of larva.
Fig. 18. Tarsus of first legs of larva.
Fig. 19. Coxae of first legs of larva.

ON THE OCCURRENCE OF THE HYPOPI OF TYROGLYPHIDS ON OTHER PARASITES

Although the occurrence of the hypopial form of tyroglyphid mites on insects has long been known and many species of such hosts have been recorded, there appears to be no record of the occurrence of these forms on other parasites.

In February 1943 I took a specimen of the beetle *Necrophorus humator* Gz. heavily infested with the deutonymphs of *Gamasus neglectus* Berl. (this is the first British record for this species of *Gamasus*). Some fifty specimens of this mite were taken from under the wing covers of the host. Every example of the *Gamasus* had attached to it great numbers of the tyroglyphid mite, *Anoetus neglectus*

Oudemans. These hypopi remained alive in a glass petri dish for some seventeen days after all the specimens of the *Gamasus* were seen to be dead. At this point the whole culture was accidentally thrown away and further observation rendered impossible.

Also in February 1943 I took an example of the somewhat uncommon species of Mallophaga, *Felicola subrostrata* (Nitz.) crawling upon a human arm. Attached to it were an adult male of *Tyroglyphus farinae* Degeer and the first hypopial form of the same species. It remained alive on the host for two and a half days, after which all the specimens were mounted for microscopic examination.

The third case is perhaps the most interesting and important. In September 1943 Miss S. M. Phillips, F.Z.S., found on a farm at Feock, Cornwall, a dying hedgehog, *Erinaceus europaeus* Linn., very heavily infested with the common hedgehog tick *Ixodes hexagonus* Leach and the hedgehog flea *Archaeopsylla erinacei erinacei* Bouche. More than a hundred specimens of the flea were collected and some dozen specimens of the tick from the great numbers present on the hedgehog, which was left in the position in which it was found. The fleas appeared to be completely covered with a fine white powder which formed a thick sediment at the bottom of the tube of 70% alcohol in which they were immersed for preservation. Upon examination this 'powder' was found to be the bodies of thousands of stage I hypopi of *Tyroglyphus farinae* Degeer. No adults were found at all. A few hypopi were found attached to the ticks, but on an average these did not number more than a dozen to each host. After exactly a week the hedgehog, now dead, was again visited and, with the exception of one or two dead fleas, none of these parasites were to be found, but a great number of the same species of tick were still alive on the carcass of the host. These ticks were noticed to be covered with an abundance of hypopi equal to that which had covered the fleas a week previously. These hypopi were still alive and, as very few were found on the body of the hedgehog, it must be supposed that either this second lot of hypopi had bred from protonymphs of *T. farinae* during the previous week or else that the hypopi from the fleas had transferred themselves to the ticks. No protonymphs had been noticed on the body of the hedgehog when it was first found, but beyond this no further evidence seems available.

The above records are interesting in several ways. Schulze (1924), as the result of her experiments on the hypopi of *T. farinae*, concludes that when removed from its natural environment the stage I hypopus dies in from one to three and a half days and under free conditions it lives only a short time. My observations, detailed above, scarcely support this, although much depends, as Schulze has shown, on the humidity of the immediate environment, the stage I hypopus soon succumbing to the effects of desiccation. It is also curious that not one example of the stage II hypopus has been observed in all this mass of material, particularly as Schulze (1924) states that a saturated atmosphere causes the stage I to change to the stage II hypopus and that this process takes from 24 to 48 hr. However, she also has found that a small number do not react to this increased moisture content unless they are subjected to it on a number of occasions. My observations on the stage I hypopus of *T. farinae* show first that it is phoretic on some ectoparasites; that in such a state of nature it is resistant to desiccation rigor for a longer time than Schulze found under laboratory conditions, or at least some strains are, and finally, that these phoretic forms of the mite frequently change their hosts, probably as the result of a simple reflex causing them to clasp anything in greater relative motion.

SOME NOTES AND OBSERVATIONS ON RADFORD'S 'GENERA AND SPECIES OF PARASITIC MITES'*

This useful list hardly contains, however, the material which its title suggests. The genera selected for the list appear to have been quite arbitrarily chosen and Radford gives no explanation for this in his introduction. In the main the arrangement appears to follow that given by Vitzhum (in Kukenthal and Krumbach's *Handbuch der Zoologie*, Dritte Band, Zweite Halfte, Erste Lief., 1931). It would have provided for easier reference had the sub-orders and families been given.

Some of the main omissions of the list should, I think, be mentioned, if only to remove the perplexity that might arise in the mind of a non-acarologist using the list for reference. No mention is made of the Tarsonemini which includes several very important parasites. None of the species of Prostigmata having parasitic larvae are included, even those parasitic upon vertebrates (Radford has evidently not intended the inclusion of any of the species of any acarine group solely parasitic on invertebrates such as the Canestriniidae). These forms would amount possibly to some 200 species and include many forms of medical and veterinary importance. None of the Demodicidae are given, although these are parasitic in all stages of their life history. Among the Sarcoptiformes neither the Acaridae (about 30 species) nor the Psoroptidae (about 15 or 16 species) appears in the list, although the Laminosioptidae, a family of only one rather obscure species, is listed.

* *Parasitology*, **35**, 58–81.

The genera of the Mesostigmata are treated very fully and accurately and as a reference to these forms Radford's list will doubtless be gratefully used by acarologists. The following points relating more particularly to the classification of the forms in this list may be advantageously treated here. The division of the genus *Laelaps* Koch into other genera is a problem that few acarologists are agreed upon. Ewing attempted a tentative division in his *A Manual of External Parasites* (Baltimore, 1929). In this work he proposes the genera *Geneiadolaelaps* type: *Laelaps barbatus* Ewing; *Echinolaelaps* type: *Laelaps echidninus* Berlese; *Atricholaelaps* type: *Laelaps reithrodontis* Ewing; *Eubrachylaelaps* Ewing type: *Laelaps hollisieri* Ewing. Radford drops all these genera and places their species in the genus *Laelaps* Koch. Ewing's genera have been accepted, at least in part, by some other authors and I think will eventually be found to correspond with real structural affinities between species within the *Laelaps* group. In this group, however, one generic name appears to me to be invalid. The generic name *Hemilaelaps* Ewing 1933 had already been used (in error) by Hull in 1918 (*Trans. Nat. Hist. Soc. North. Durh. and Newcastle*, **5** (new series), 1918), for the *Eulaelaps* group of species which by mistake he referred to the genus *Haemolaelaps* Hirst and which he wrote in error Hemilaelaps. I wrote informing Dr Ewing of this in January 1942, but the mistake does not seem to have been rectified, possibly my communication was not received by him owing to the wartime conditions prevailing. I therefore now propose the generic name *Ellsworthia* (from Ewing's christian name, as a genus *Ewingia* is already preoccupied (Pearse, 1929) and to combine this with laelaps to form a compound generic name is objectionable) to take the place of *Hemilaelaps* Ewing 1933.

An error in Radford's list may be corrected here. He gives the genus *Eubrachylaelaps* to Fonesca 1935 instead of to Ewing 1929 as quoted above—and incidentally removes the type of the genus *E. hollisteri* Ewing 1925 to the genus *Laelaps*!

To the genus *Laelaps* itself should be added *L. stegemani* Hefley 1935 (*J. Kansas Ent. Soc.* **8**, no. 1), found on the common skunk *Mephitis nigra*.

To the genus *Eulaelaps* should be added *E. stabularis* var. *proximus* Berl. 1903 (*Redia*, **1**) from the lemming, a form which appears to be quite distinct.

To *Ichoronyssus* add *I. albato-affinis* Ouds. 1902 (*Tijdsch. Nederl. Dierck. Ver.* **8** (2)). The hosts are given as '*Mus rattus* and *Arvicola arvalis*'. The entry for *Ichoronyssus spinosus* Ouds. should read *I. spinosus* Ouds. 1902 (*Tiddschr. Nederl. Dierck. Ver.* **7** (2)) and the host is there given as '*Vespertilio murinus*'.

After the genus *Listrophorus* Pagens should be inserted the genus *Euryzonus* Trouessart 1918 (*Bull. Soc. Zool.* **42**) with the species *ventricosus* Trsst. found on *Sigmodon* in Brazil. Following this the genus *Chirodiscoides* Hirst, 1917, must become *Campylochirus* Trouessart, 1893, as Ewing has shown (*Manual of External Parasites*, 1919, p. 44).

I have to thank Dr F. J. Killington for the opportunity to study and describe the new *Leptus* larva, and my pupil, Miss Stella Phillips, F.Z.S., for the new species of *Myobia* and for the collection of a great amount of material from a dying hedgehog, on which the greater part of the notes on the association of hypopi with other parasites are based.

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THE ECOLOGY OF THE SHEEP TICK, *IXODES RICINUS* L.

THE SEASONAL ACTIVITY IN BRITAIN WITH PARTICULAR REFERENCE TO NORTHERN ENGLAND

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(With 3 Figures in the Text)

CONTENTS

	PAGE
1. Seasonal activity of female *Ixodes ricinus* on sheep	142
(i) Introduction	142
(ii) Observations in northern England	142
(*a*) Methods	142
(*b*) Seasonal activity in relation to temperature	143
(*c*) Seasonal activity in relation to solar radiation	144
(*d*) Seasonal activity in relation to evaporativity and humidity	144
(iii) Observations in northern Scotland	148
2. Seasonal activity of male, nymphal and larval *Ixodes ricinus* as shown by blanket dragging	148
(i) Method	148
(ii) Manipulation of blanket data	148
(iii) Seasonal activity of males and nymphs	149
(iv) Seasonal activity of larvae	149
3. Discussion	150
4. Summary	151
References	152

1. SEASONAL ACTIVITY OF FEMALE *IXODES RICINUS* ON SHEEP

(i) Introduction

On the seasonal incidence of female *Ixodes ricinus* on sheep in Britain, MacLeod (1939) gives extensive data of his own and covers all the relevant previous work. His findings summarized are: In Argyllshire, Perthshire, Selkirk and Northumberland 'tick incidence is greatest in spring and decreases markedly in early summer. There is evidence of a similar periodicity in North Wales'. Evidence is also given of 'a slight recrudescence of activity in autumn, and of the almost complete absence of activity in winter'. 'The spring curves of activity terminated in early May in Argyllshire, in the middle of May in Perthshire, and towards the end of May in the Borders and Northumberland. The seasonal activity was found to be related to the air temperature (shade), expressed as the weekly average of maximum temperatures. The limits of air temperature range corresponding to active tick infestation are 45 and 60° F., average weekly maximum,' a range which corresponds with spring and autumn temperatures in this country. 'The curve of tick infestation during 1937 on a moor in N.E. Scotland was found to differ markedly from the normal,' and 'the possible explanations of the discrepancy' were discussed (see later in this paper).

In his discussion, MacLeod states: 'The known facts of seasonal incidence would thus seem to be most readily reconcilable with the theory of temperature control of tick activity.... Circumstantial evidence in favour of activity being limited to a given temperature range is found in the seasonal habits of ticks in different climatic regions. Thus, in North Africa, the southernmost limit of distribution of *I. ricinus*, the species is active only in winter, whereas in North Russia, the northernmost limit, it is active only in summer.'

The term 'tick activity', as used in the present paper, does not refer to the physiological condition of the tick, but merely to the periods of its infestation of the sheep host, which correspond with the periods during which it is found, in blanketing, on the vegetation tips, i.e. 'active on the ground'.

(ii) Observations in northern England

(*a*) *Methods*

Counts of female ticks on 10 hoggs (one-year-old sheep) have been carried out weekly on two hill farms, Crag in south-western Cumberland and Hethpool in north-eastern Northumberland, during the 4 years 1940–3. These situations were chosen mainly because they gave something approaching the widest possible difference in climatic conditions obtaining in northern England: at Crag, the humid

mild climate of the west; at Hethpool, the drier, cooler climate of the east. The Crag hill-grazing is composed of rough bent-type grasses, bog-myrtle, heather and bracken, and the land ranges from wet and boggy to dry and rocky; the Hethpool hill-grazing is composed of rough bent-type grasses and bracken, and the land is not boggy, being mainly drier than at Crag. Herdwick sheep are kept on Crag, and Cheviot on Hethpool.

The most important meteorological factors in the case of *I. ricinus* are temperature and humidity. Various records were kept continuously for 2–4 years at stations situated about the middle height of the grazings on the two farms. In the customary 'screen', a thermohygrograph was installed, together with maximum-and-minimum and wet-and-dry-bulb thermometers for checking purposes. In the open, near each screen, a Piche evaporimeter (atmometers being unobtainable during the war) and a maximum thermometer were arranged about the average height of the tips of the vegetation. The latter gave some idea of the relative amounts of solar radiation on the two farms.

(*b*) *Seasonal activity in relation to temperature*

In Fig. 1 the curves of weekly average tick counts on sheep and weekly averages of daily maximum temperature in the screen (i.e. shade temperature) may be compared. Double-dipping operations under the Sheep Scab Order prevented tick counting on sheep at Hethpool in the early part of the 'spring' season of 1941 and 1942. Fortunately, however, the counts round about peak activity were obtained. Owing to farming difficulties one and two counts respectively in 1942 and 1943 could not be made at Crag. These omissions may affect both the level of the peak and its date:

1942		1943	
27 Apr.	40·6 ticks	1 May	29·7 ticks
4 May	73·7 ,,	8	Omitted
11	84·3 ,,	15	Omitted
18	Omitted	22	69·5 ticks
25	72·7 ticks	29	54·9 ,,

Clearly peaks could be higher in both years—almost certainly in 1943, and the peak could have been 1 week later in 1942 and 1 week earlier in 1943 than Fig. 1 suggests.

In counting at intervals, the true peak may be anywhere within plus or minus half an interval of the recorded peak. This is important in the case of Moore's counts with intervals varying from 1 week on some farms to 4 weeks on others (Fig. 2).

Before proceeding to analyse the data on *seasonal* activity in relation to temperature, that aspect of Fig. 1 suggesting the possible comparison of *annual* levels of tick incidence must be dismissed. Obviously quite large variations in level of spring peaks occur on both farms:

	Crag Farm	Hethpool Farm
1940	103·7 ticks per sheep	83·4 ticks per sheep
1941	85·7 ,,	25·9 ,,
1942	84·3 ,,	50·3 ,,
1943	69·5 ,,	51·6 ,,

But apart from 4 years being too short a period from which to draw conclusions, several factors render the above data useless for the purpose: (1) A different set of 10 sheep were used in each year, and two sets of 10 sheep going on the same grazing at the same time can have and usually do have very wide differences in mean infestation (see Milne, 1943). (2) On Hethpool a certain amount of serial dipping of sheep to reduce the tick population of the grazing has been in progress since 1937. (3) On Crag, no serial dipping has been done, but the peaks for 1942 and 1943 could have been higher (see last paragraph); even so, the difference between means of 103·7 and 69·5 ticks on 10 sheep is not significant (see Milne, 1943, Table 2).

Hethpool. On this farm there is substantial agreement with MacLeod's findings for 'spring' activity in the 'Borders and Northumberland', viz. activity comes to an end towards the close of May or the beginning of June at about the 60° F. level. The curves of activity seem to follow the temperature conditions. For instance, air temperatures rose earlier in spring (April–May) 1940 and 1943 than in spring 1941 and 1942, ultimately passing over 60° F. in May in the former two years and not until June in the latter two. This is reflected in the times of peak activity which were earlier in the former case (11 April 1940 and 5 April 1943) than in the latter (5 May 1941 and 27 April 1942). With the exception of 1943, which followed a record mild winter and a correspondingly early commencement of spring, the peaks all occurred about the 50° F. level. The early spring peak in 1943 was typical of north-eastern Northumberland as was shown by enquiries to shepherds and by the following average tick counts on a farm 20 miles south of Hethpool: 9 April, 75·2 ticks per sheep; 16 April, 106·9; 23 April, 69·0; 30 April, 48·6; 7 May, 47·0; 14 May, 21·1; 21 May, 16·0; 28 May, 10·2.

A peculiar feature of activity at Hethpool is that no autumn recrudescence of activity was observed in any of the 4 years: from the end of June onwards, the average infestation was always less than one tick per sheep. This is atypical for Northumberland. Two miles up the valley, and in neighbouring valleys 15–20 miles south, the usual autumn recrudescence began about mid-August with peak in September and ended about mid-October. An unpublished report (Cameron, 1942) records the absence of autumn recrudescence of female ticks in Ettrick

valley during 1938 and 1939; MacLeod had also shown this previously for Ettrick. Since average maximum temperatures pass down through the 60–45° F. range during autumn, the absence of activity then is at variance with the theory of temperature control described in the introduction.

Crag. Invariably on this farm an autumn recrudescence occurs in September with peak varying from about one-third to more than one-half the level of the spring peak. The spring peaks occurred on 6 May 1940 and 11 (18) May 1943; 19 May 1941 and 22 (15) May 1942. (The figures in brackets are possible alternatives—see para. 1 of this section.) Again, the indication is that spring peaks were probably earlier in 1940 and 1943 than in 1941 and 1942 for temperature reasons. These peaks occurred at temperatures ranging from 55 to 67° F. Activity did not stop altogether in summer, was never lower than 1·5 ticks per sheep, and was below 5 ticks per sheep for short periods only, viz.

1940	24 June–29 July, i.e. about 5 weeks
1941	7 July–4 Aug., i.e. about 4 weeks
1942	13 July–17 Aug., i.e. about 5 weeks
1943	19 June–3 July, i.e. slightly more than 2 weeks

The most striking fact, however, was that more than half the annual activity occurred at temperatures *above* the 60° F. level, i.e. 60–70° F. weekly average maximum shade temperature. Indeed, in the autumn, activity started to wane when temperatures passed below the 60° F. level (see Fig. 1). In general, temperatures were no higher when the average tick counts were below 5 than they were during lengthy periods of the higher activity before and after. Thus average maximum temperature alone is not the reason why activity reaches a summer minimum on Crag.

MacLeod's temperature records consisted of data observed, in some instances, on the actual farms where tick counts were made, and, in others, at the official meteorological stations nearest to the farms. In both cases he found the upper limit of activity to be 60° F. average maximum shade temperature. The findings on Crag are again at variance with MacLeod's theory.

During the years 1940, 1941 and 1942 the curves for average temperature at Crag were generally at a higher level throughout the year than those at Hethpool. The data is incomplete for 1943. Means of weekly averages of daily maximum temperature in degrees F. were:

Year		Period	Crag	Hethpool
1940	8 Apr.–11 Nov.	31 weeks	62·23	59·29
1941	24 Mar.–24 Nov.	35 ,,	58·70	55·79
1942	6 Apr.–2 Nov.	30 ,,	60·97	57·09

(c) *Seasonal activity in relation to solar radiation*

The weekly average solar radiation curve (see Fig. 1) follows the curve for weekly average of daily maximum shade temperature—at a higher temperature level, of course, and higher at Crag than at Hethpool in 1942, the only year for which there are complete records on both farms. The highest average 'solar radiation' recorded was 85° F., which occurred, among other times, during the autumn peak of 1942 on Crag. Although the present 'solar radiation' values are merely records of the temperature to which the particular kind of thermometer used was raised in the direct rays of the sun, ticks are seemingly able to withstand high heat effects when active. In blanket dragging, ticks have been observed in hot sunlight at the tops of long grass stems too narrow to give them shade. Nothing is known about how long the tick can withstand high insolation.

(d) *Seasonal activity in relation to evaporativity and humidity*

Fig. 1 shows no obvious correlation between evaporativity and seasonal activity on either farm. In all cases evaporativity has a more or less uniform decline from spring to autumn. Average evaporativity was higher on Crag in 1941, but higher on Hethpool in 1942, viz.

Year		Period	Mean of weekly totals of evaporation in c.c. Crag	Hethpool
1941	12 May–20 Oct.	23 weeks	20·99	17·24
1942	20 Apr.–2 Nov.	28 ,,	20·25	23·52

MacLeod (1939) states that 'under summer conditions of temperature, the unfed tick requires for survival (*in vitro*) an aqueous vapour tension equivalent to over 80 % saturation'. In another place (MacLeod, 1938) he states 'except during winter low temperatures neither the unfed nor the gorged tick will survive more than a few days under ordinary air conditions in this country'. (MacLeod, 1934, 1935).... 'Humidity has no effect on activity beyond the indirect one of affecting duration of life.' For the unfed female Lees (1944) shows 92 % R.H. (range 86–96 % R.H. in 10 individuals) to be the equilibrium humidity at ordinary temperatures, i.e. above or below this humidity the tick takes in or gives out water.

For the years 1940–3 the (thermohygrograph) relative humidity data at Hethpool are complete, but only for the years 1941 and 1942 at Crag. The daily maximum and minimum humidities and the number of hours each day at less than 80 % and more than 92 % R.H. have been extracted from the 1941 and 1942 charts. The monthly averages for 1942 are cited as typical in Table 1 with only 'hours at less than 80 %' for 1941.

There is an almost continuous diurnal fluctuation in % R.H. related to the diurnal fluctuation in air

temperature. Except during rain, mist, fog, etc., humidity falls to its lowest point during the heat of the day and rises to its highest during the cool of the night. Daylight humidity very seldom exceeds humidity in the hours of darkness. Thus in Table 1 the higher humidities including the maximum may be taken as occurring in the hours round midnight, and the lower humidities including the minimum in the hours round midday.

Between the end of March and the beginning of November, the extreme limits of appreciable tick activity for the year in northern England, the only trend in humidities is a more or less steady rise in the minimum, equal to 10–15 % R.H. in 1942. This is naturally coupled with a decrease in the hours per day at less than 80 % R.H. and also agrees with the parallel increase in evaporativity noted above.

(2) At less than 80 % R.H. the hours per day at Hethpool are generally higher than at Crag. On the average there are twice as many hours on the former as on the latter (1942 data).

(3) In June, July and August, the months when tick activity in north-eastern Northumberland generally has practically ceased, the number of hours per day at less than 80 % R.H. is more than four times greater at Hethpool than at Crag. The averages for the 3 months are 7·83 hr. per day at Hethpool and 1·83 hr. at Crag (1942 data).

Thus, on the standard of MacLeod's laboratory work, air-humidity conditions (macro-climate) are consistently and continuously more favourable to ticks throughout the year at Crag.

At the vegetation tips, the 'active' position, the tick experiences temperature-humidity conditions

Table 1. *Humidity data from Hethpool Farm (A) and Crag Farm (B).*

1942 Month	Average of daily R.H. % Max. A	Max. B	Min. A	Min. B	Average of daily difference between max. and min. A	B	Hours per day at < 80 % R.H. A	B	> 92 % R.H A	B
April (31 Mar.–27 Apr.)	95·6	93·2	59·5	70·1	36·1	23·1	10·3	7·2	5·9	8·3
May (28 Apr.–1 June)	95·5	93·3	58·7	68·0	36·8	25·3	10·9	9·0	5·0	8·6
June (2–29 June)	95·0	95·3	63·5	78·1	31·5	17·2	10·7	3·4	5·5	9·7
July (30 June–3 Aug.)	96·9	94·1	66·3	83·9	30·6	10·2	8·6	1·6	7·4	9·3
August (4–31 Aug.)	96·6	93·1	73·8	83·8	22·8	9·3	4·2	0·6	9·4	8·4
September (1–28 Sept.)	96·3	93·2	69·0	82·8	27·3	10·4	6·4	0·8	6·3	3·7
October (29 Sept.–2 Nov.)	95·8	92·5	73·5	82·3	22·3	10·2	3·7	0·7	6·5	2·9
Average (31 Mar–2 Nov.)	96·0	93·5	66·3	78·4	29·6	15·1	7·8	3·3	6·6	7·3

1941 (month) ...	Farm	Apr.	May	June	July	Aug.	Sept.	Oct.
Hours per day at < 80 % R.H.	A	6·8	9·9	9·2	7·8	6·3	4·2	5·1
	B	10·2	13·5*	—	0·0*	0·4	0·0	1·7

* 22 May–6 July no data due to shrew having cut through hair of hygrograph.

Humidity by itself is, therefore, not directly correlated with the *rhythm* of activity on either farm. Autumn peaks are invariably less than spring peaks and humidities higher in autumn than in spring. The connexion, if any, between these facts is unknown.

Since the climate of the west is wetter, the fact that the average daily maxima at Crag are the smaller (see Table 1) is probably due to an idiosyncrasy of the thermohygrograph used there. It is safe to say that on both farms humidity rises to 95 % R.H. or over almost every night. Apart from this, humidity conditions are different on the two farms:

(1) Minimum daily humidity is consistently lower at Hethpool. On the average it is well under 70 % R.H. at Hethpool while just under 80 % at Crag. Consequently the diurnal humidity fluctuation is greater at Hethpool, being on the average twice as great as at Crag. Ecologically, rate and range of fluctuation are very important.

approximating those of the macro-climate. Because of the rare chance of a host passing *exactly* within reach, an active tick must spend long periods at the vegetation tips. Now, on a hill farm, the tick is practically absent on the areas where sheep spend the night (Milne, 1944). Hence, for success (the sheep being the chief host), most of the ticks are obliged to spend these long periods at the vegetation tips *during daylight* when humidities are at their lowest (see Table 1). Since high temperatures have less ill-effect when accompanied by the higher humidities (MacLeod, 1935), the encroachment of activity into summer at Crag (see Fig. 1) may be partly if not wholly due to higher humidities for longer periods of daylight (see preceding paragraph). This is speculative, but the activity of an organism with such a narrow humidity survival range is likely to be more influenced by humidity than MacLeod, without experimenting (see 1935, pp. 137–9), seems to have thought (1938, p. 1247; 1939*a*, p. 100).

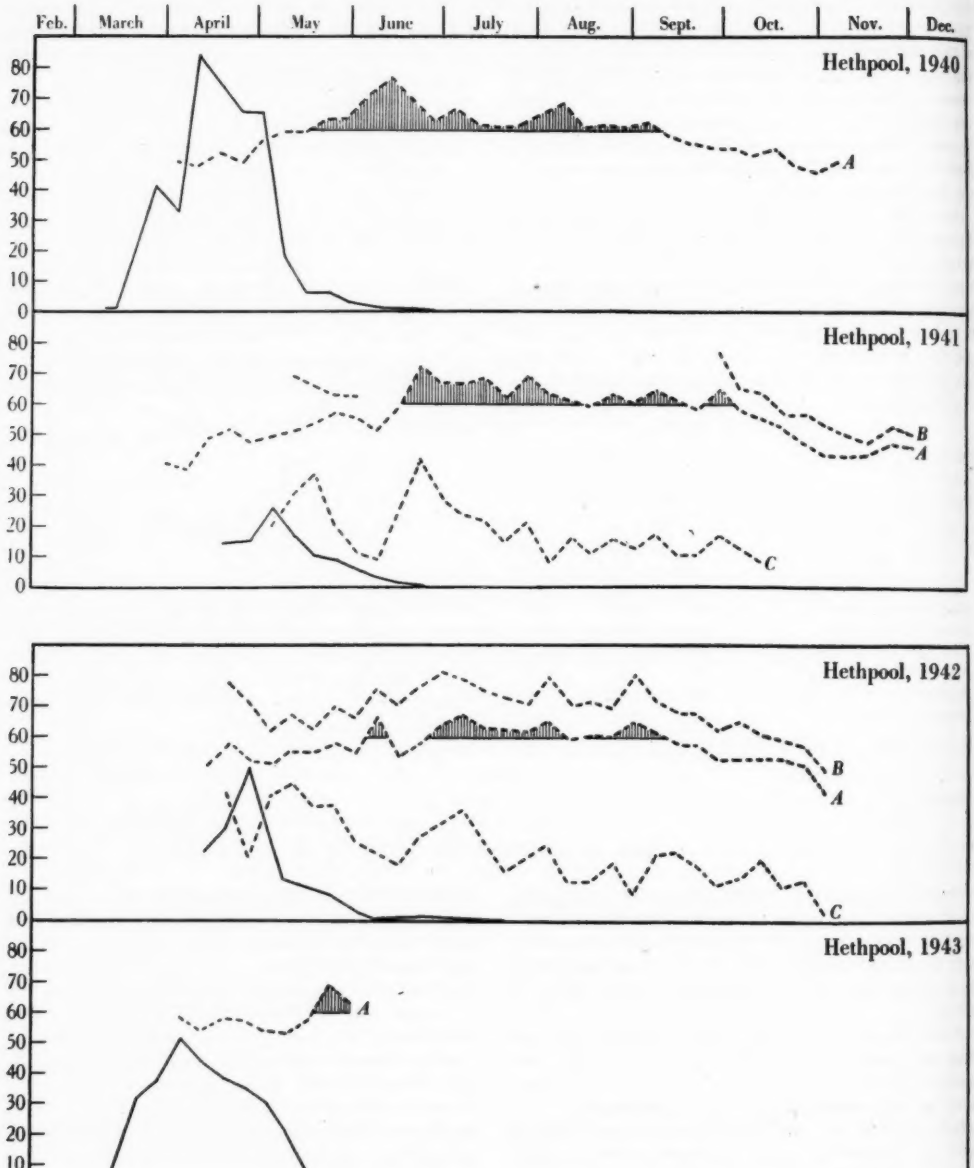

Fig. 1. Tick activity curves in north-eastern Northumberland (Hethpool) and south-western Cumberland (Crag). Unbroken lines show average weekly female tick infestation per sheep (in groups of 10 sheep). Broken lines show (*A*) weekly average of daily maximum shade temperature °F., with shaded portion indicating over 60°F., (*B*) weekly average of daily 'solar radiation' maximum temperature °F., and (*C*) weekly totals of evaporation in c.c. (i.e. evaporativity).

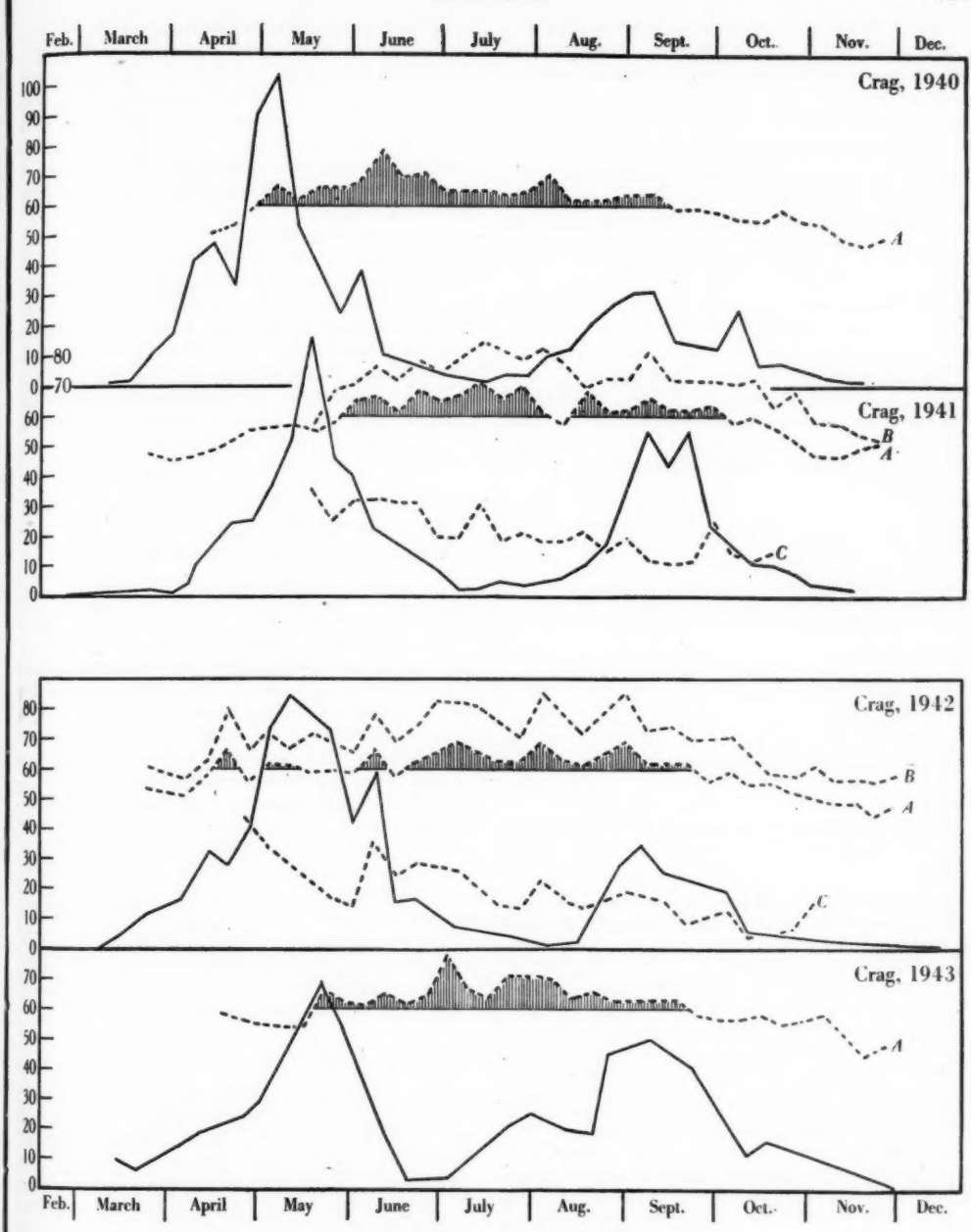

Fig. 1. For legend see opposite.

The only certain conclusion is that the influence of temperature-humidity interaction on activity deserves to be investigated in laboratory and field.

(iii) Observations in northern Scotland

Concerning the single annual peak, in summer, at the Hill o'Fare in Aberdeenshire (Hendrick, Moore & Morison, 1938), MacLeod (1939) gives reasons for thinking that conditions on this moor are abnormal.

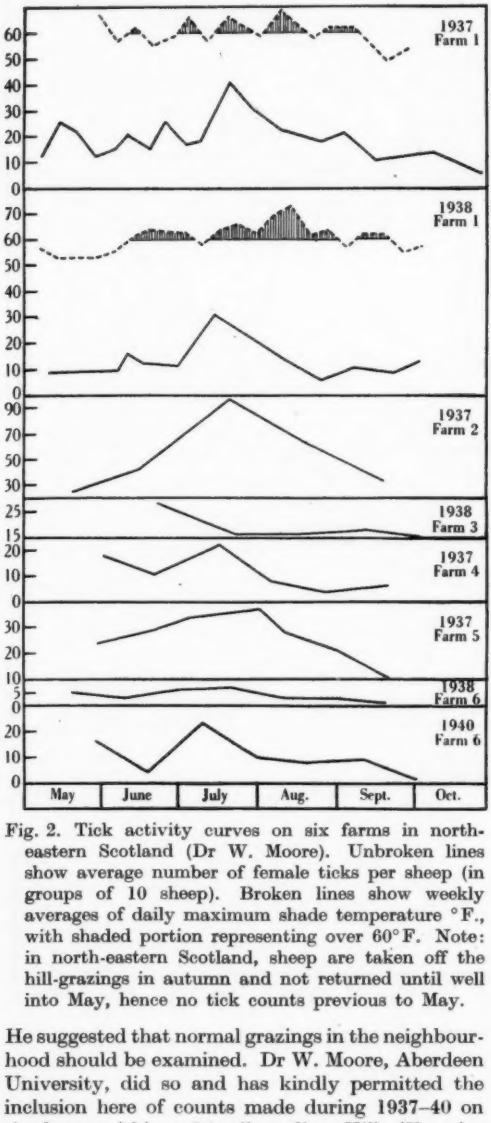

Fig. 2. Tick activity curves on six farms in north-eastern Scotland (Dr W. Moore). Unbroken lines show average number of female ticks per sheep (in groups of 10 sheep). Broken lines show weekly averages of daily maximum shade temperature °F., with shaded portion representing over 60°F. Note: in north-eastern Scotland, sheep are taken off the hill-grazings in autumn and not returned until well into May, hence no tick counts previous to May.

He suggested that normal grazings in the neighbourhood should be examined. Dr W. Moore, Aberdeen University, did so and has kindly permitted the inclusion here of counts made during 1937–40 on six farms within a 15 mile radius, Hill o'Fare included. The 1937 and 1938 data for farm 1 has already been published (Moore, 1938, 1939). As a whole the data show (Fig. 2) that here the female tick is most active in June-July-August with peak generally in July, there being no spring or autumn peak. According to MacLeod (1939, Fig. 12), the monthly averages of maximum temperature in Aberdeenshire vary between 60 and 65° F. in June, July and August.

In a private letter (1943) Moore also gives evidence (not yet fully confirmed) of activity in Nairnshire coming between that of Aberdeenshire and that of the Borders-cum-Northumberland, viz. activity rising to a peak in late May or early June, dying down at the end of June, and recommencing again in early August. This is reminiscent of activity at Crag.

The evidence from north-eastern Scotland again shows that MacLeod's theory of temperature control of activity is not universally applicable in Britain.

2. SEASONAL ACTIVITY OF MALE, NYMPHAL AND LARVAL *IXODES RICINUS* AS SHOWN BY BLANKET-DRAGGING

(i) Method

Although MacLeod (1932, 1939) gives a few nymph counts on sheep, the intensive study of the seasonal activity of any stage except the female by counts on sheep is impracticable. Hence the problem has been approached by the blanket-dragging method (see Milne, 1943). Simultaneous triple blanket-draggings for all stages of the tick were carried out around noon on several different areas of Hethpool during the week preceding each female tick count on sheep, viz.

Year	Period	No. of areas dragged	Average no. of days of dragging per week	Average no. of drags per week
1941	16 Apr.–30 June	3	5	45
1942	1 Apr.–30 June	5	3	45
1943	6 Apr.–26 May	4	3	36

(ii) Manipulation of blanket data

The weekly drag averages produce a more irregular activity curve than do weekly average counts on sheep. The reason is that the sheep, being constantly on the ground, is experiencing all the activity possibilities during the week (168 hours) while the blanket samples only those activities occurring during $4\frac{1}{2}$–$7\frac{1}{2}$ hr. of the week always at the same time of day. In the latter case, estimated averages of weekly activity must necessarily deviate from the actual as widely as may weather conditions around noon in any week. Table 2 is a small section of the 1943 data illustrating this point.

The figures in brackets are calculated by 'sliding the mean', i.e. taking the mean of the original values three at a time and moving down one for each fresh calculation. This expedient provides smooth curves for the blanket data which facilitate graphic comparison. In the case of the counts of females on sheep the process has displaced the peak 1 week. This is bound to happen when the curve falls away faster on one side of the peak. It makes doubtful the comparison of curves differing widely in shape. The *raw data* curves for different tick stages *on the blanket* do not differ widely—see those for females, males and nymphs in Table 2 where the rising and falling in numbers from week to week is coincident (governed by meteorological factors). Hence, when comparison of *relative* positions of peak times of different stages is confined to blanket data, any displacement of peak due to sliding the mean is unimportant because it will have the same extent and direction for each stage. Blanket data, however, cannot be depended upon to give *true* peak times. For instance, the peak time for females occurred at the same date on sheep and blanket in 1942, but was 1 week later on the blanket in 1941 and 3 weeks later in 1943. Sheep counts, of course, give the true peak time. The aberrations in the case of the blanket counts arise from blanketing giving a poorer sample of weekly activity than sheep.

(iii) Seasonal activity of males and nymphs

MacLeod (1932, Chart III, and 1939, Fig. 5) gives spring (4 examples) and autumn (2 examples) activity curves for both females and nymphs *on sheep*. These plainly show that, although nymphs are more numerous than females, the curves for the two stages are similar and concurrent, the peaks occurring at approximately the same dates. He also points out (MacLeod, 1932) that males and females in almost equal but small numbers appear a few weeks before nymphs in spring; yet the *bulk* of the activity of all three is concurrent. Since the present blanket-dragging data confirms MacLeod's findings, i.e. that males, females and nymphs have similar and concurrent activity curves in spring, the unwieldy blanket data need not be published here. Observations on sheep also confirm that nymphs make their first appearance a few weeks later than males and females in spring.

(iv) Seasonal activity of larvae

On the seasonal activity of larvae, where female activity occurred in spring and autumn, MacLeod (1932) has one brief sentence: 'Larvae were found throughout the tick season, but appeared to be most numerous in May–June,' whereas females and nymphs were most numerous in April-May. Since the present work amplifies and makes a minor correction in this statement, the results are given in detail.

Table 2. *Weekly averages of tick counts per sheep and per blanket drag (section of* 1943 *data)*

Week ending	On sheep: females	On blanket: females	On blanket: males	On blanket: nymphs
15 Mar.	8·7 —	— —	— —	— —
22	31·9 (26·2)	— —	— —	— —
29	37·9 (40·5)	— —	— —	— —
5 Apr.	51·6 (44·5)	— —	— —	— —
12	44·0 (44·8)	0·17 —	0·18 —	2·37 —
19	38·7 (39·4)	1·28 (0·69)	0·92 (0·52)	21·89 (11·81)
26	35·4 (34·9)	0·61 (0·81)	0·47 (0·56)	11·17 (14·27)
3 May	30·5 (29·2)	0·54 (0·73)	0·29 (0·55)	9·75 (11·47)
10	21·8 (19·5)	1·04 (0·57)	0·87 (0·43)	13·50 (8·42)
17	6·1 (9·9)	0·12 (0·43)	0·12 (0·41)	1·96 (6·16)
24	1·8 (3·0)	0·14 (0·15)	0·22 (0·20)	3·03 (3·24)
31	1·2 —	0·19 —	0·25 —	4·75 —

On the blanket larvae are found in clusters each arising from the egg-laying of one female. Because of the impracticability of counting individual larvae in blanket-dragging, only the number of clusters was recorded. Table 3 shows the weekly average numbers of females and larval clusters per blanket drag.

For easy comparison Fig. 3 shows smooth curves calculated from the figures in Table 3 by sliding the mean and adjusting the individual resulting values in proportion, so that the peak value is 15·0 in every case. Plainly the curves differ widely, and the larval cluster peak was later than the female by 5 weeks in 1941, 4 weeks in 1942 and 3 weeks in 1943. Fig. 3 also indicates that the larval curve begins later than the female. This is confirmed by the dates of first encounters with either stage, viz.

	1940	1941	1942	1943
Blanket sampling commenced	25 Mar.	16 Apr.	1 Apr.	6 Apr.
First encountered females	26 Mar.	16 Apr.	7 Apr.	6 Apr.
First encountered larvae	2 May	21 Apr.	21 Apr.	19 Apr.

Table 3. *Weekly average numbers of female ticks and larval clusters per blanket drag*

1941			1942			1943		
Date	Females	Larval clusters	Date	Females	Larval clusters	Date	Females	Larval clusters
21 Apr.	0·233	0·067	13 Apr.	0·029	0·000	12 Apr.	0·167	0·000
28	0·071	0·000	20	0·400	0·000	19	1·278	0·000
5 May	0·167	0·042	27	0·500	0·033	26	0·614	0·056
12	0·205	0·128	4 May	0·200	0·000	3 May	0·542	0·000
19	0·125	0·125	11	0·200	0·000	10	1·042	0·042
26	0·152	0·152	18	0·200	0·022	17	0·125	0·042
2 June	0·157	0·020	25	0·013	0·133	24	0·139	0·111
9	0·061	0·182	1 June	0·007	0·033	31	0·194	0·000
16	0·000	0·167	8	0·022	0·000			
23	0·051	0·103	15	0·007	0·000			
30	0·028	0·111	22	0·027	0·040			
			29	0·000	0·000			

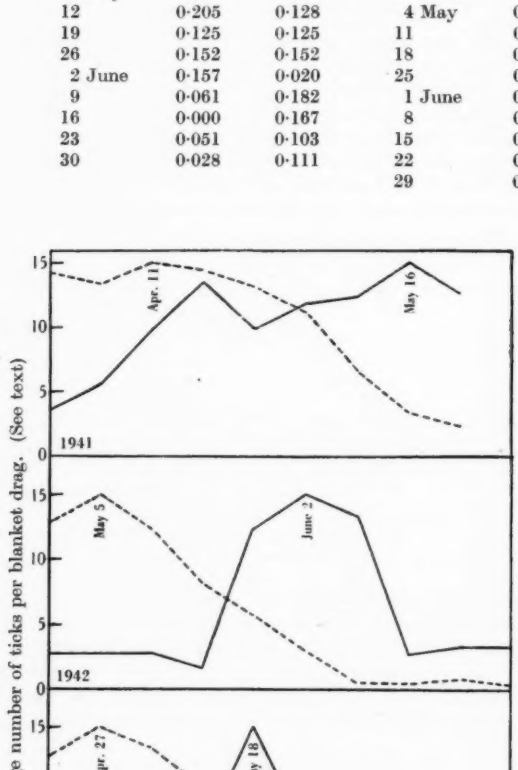

Fig. 3. Portions of spring activity curves showing peaks. Data from blanket-dragging. Broken line for female ticks; unbroken line for larvae.

Hence at Hethpool larvae were not found 'throughout the tick season', but their peak is about 1 month later than the other stages in spring.

3. DISCUSSION

Various theories on seasonal activity have been put forward:

The 'two-brood' theory (Wheler, 1899) is described (and dismissed) by MacLeod (1939) as the belief 'that the spring and autumn ticks represented two successive broods, those ticks which gorged in the spring completing their development and attacking sheep in their next stage in autumn'. Activity in spring must, according to this theory, finally cease because all the ticks ready to feed have been fed. Obviously the theory does not explain seasonal activity rhythms in Britain as a whole, while the absence of autumn activity at Hethpool and Ettrick, both with 'normal' spring activities, demands the assumption that summer micro-climate conditions retard development in these places. Nevertheless, the two-brood theory contains the germ of an explanation why autumn peaks, where they occur, are invariably considerably lower than spring peaks. Although development proceeds faster at summer than at winter *air* temperatures (MacLeod, 1934), it must be remembered that the micro-climate in which eggs and gorged ticks exist (i.e. the deeper layers of the vegetation) is cooler in summer and warmer in winter than the air. Again, the time from autumn peak to spring peak (September–May, 8 months) is twice as long as from spring peak to autumn peak (May–September, 4 months). Winter micro-climate duration-temperature conditions may permit *all* the ticks fed in autumn to complete their development in time for the following spring season, while those conditions for summer may permit only a *fraction* of the ticks fed in spring (i.e. those fed in the first part of the season) to complete their development in time for the following autumn season. Thus where autumn activities are half the weight of spring: (*a*) each autumn activity would be composed of ticks developing from the first half of the previous spring activity; (*b*) each spring activity would be

composed of ticks developing from the second half of the previous spring activity plus the whole of the previous autumn activity.

The diapause theory (Falke, 1931, Totze, 1933) deals only with the winter phase of inactivity. MacLeod (1935) states that, in Britain at least, there is no indication 'of the over-wintering torpor of unfed ticks described by Totze or of gorged ticks, by Falke'. Dr E. T. Burtt (Agricultural Research Council Unit of Insect Physiology), working on ticks from the north of England, thinks MacLeod may be mistaken about British *I. ricinus*, but has not confirmed this fully yet.

The temperature control theory (MacLeod, 1939) was perfectly reasonable when based on the not inconsiderable data then available. It fails to stand the test of additional data.

The theory of different physiological races (MacLeod, 1939) was a tentative suggestion concerning the 1937 curve for Hill o'Fare, viz. 'If the same result can be demonstrated for ticks on normal grazings in the N.E. of Scotland, the evidence will justify the assumption that a physiologically different race of ticks exists in the area.' This begs the question, Why would there be a physiologically different race in north-eastern Scotland? Nevertheless, this theory cannot be dismissed without investigation.

The writer believes that all four theories may contribute something to the truth and, in addition, that temperature-cum-humidity *in the micro-climate* deserves further investigation. At the vegetation tips (the 'active' position) the tick experiences temperature-humidity conditions approaching macro-climatic. Yet its ability to function there may be influenced by the micro-climatic gradient from the tips downwards. Micro-climatic changes per unit of descent will vary as much as the physical character of the micro-habitat varies (cf. Milne, 1944). Blanketing has shown ticks active when humidity at the vegetation tips was 40 % R.H. in hot sunshine. How long they could endure these conditions is unknown, but if they could endure all the daylight hours they could make up water loss in the same position at night when humidity almost invariably rises over 95 % R.H. and temperatures are much lower. Probably, however, in these conditions ticks are obliged to make periodic descents in daylight to a point in the micro-climate where humidity and temperature conditions are such that water loss can be made good. If macro-climatic temperature is too high and humidity too low for too long a daylight period (cf. Hethpool as opposed to Crag in summer), and suitable conditions are too deep down in the micro-climate, one can imagine ticks being unable to withstand the labour and stresses on their system occasioned by frequent descents and ascents. On the other hand, where the micro-climatic gradient is steep, ticks may find suitable conditions at a point not too far below the tips for them to 'make their grab', i.e. remain active.

Whatever may be the causes of differences in seasonal rhythms, a knowledge of activity curves in any area is a prerequisite to the practice of control by serial dipping of sheep.

4. SUMMARY

A survey of tick activity curve data for Britain shows:

There are usually two peaks of female activity per annum, one peak in 'spring' and a lesser one in 'autumn', the date limits of the 'seasons' being slightly elastic. For a greater or lesser period in summer female-tick activity is low or practically absent, while in winter it is practically absent.

There are exceptions to the biannual type of female activity. In three areas, only one annual peak occurs. At Hethpool, north-eastern Northumberland, and in Ettrick Valley, southern Scotland, this single peak is in April–May; in the Hill o'Fare area, north-eastern Scotland, it is in July.

The theory of temperature control of activity (the limits being the range 45–60° F. average weekly maximum shade temperature, i.e. corresponding to spring and autumn conditions in Britain) does not stand up to the following facts: At Hethpool, following a spring female activity confined to the range 45–60° F., there was no autumn recrudescence when temperatures again passed through the same range. About half the annual female activity at Crag, south-western Cumberland, and practically all in the Hill o'Fare area occurred at temperatures of 60–70° F. and 60–65° F. respectively.

Solar radiation, evaporativity and humidity, as well as temperatures, of the macro-climate were investigated in relation to female activity. The tentative conclusion is that the duration of humidity level during daylight, in conjunction with temperature, may partly decide the amount and extent of activity in summer.

In spring, where spring activity obtains, males and females appear in *small* numbers a few weeks before nymphs. The *bulk* of male, female and nymphal spring activity, however, is concurrent, the curves having peaks at approximately the same date.

Again, where spring activity obtains, spring larval activity commences later than that of males, females and nymphs, the peak being about 1 month later.

Theories of seasonal activity are discussed.

The writer is deeply indebted to Dr W. Moore for his generosity in allowing to be published here for the first time the graphs of farms 2, 3 and 4 in Fig. 2. Thanks are also due to Dr A. E. Cameron, Edinburgh

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THE ECOLOGY OF THE SHEEP TICK, *IXODES RICINUS* L.

HOST AVAILABILITY AND SEASONAL ACTIVITY

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(With 1 Figure in the Text)

CONTENTS

	PAGE
1. Introduction	153
2. Methods	153
3. Delayed stocking with sheep and the activity incidence curve ...	154
4. Discussion	156
5. Practical conclusions	157
6. Summary	157
References	157

1. INTRODUCTION

In certain areas of Britain, *Ixodes ricinus* infests sheep heavily in spring and autumn, but is practically absent from sheep in summer and winter (MacLeod, 1939; Milne, 1944; and others). Wheler (1899) suggested the 'two-brood' theory as an explanation of this rhythm. MacLeod (1939) described the theory as 'the generally accepted belief that the spring and autumn ticks represented two successive broods, those ticks which gorged in the spring completing their development during the summer and attacking sheep in their next stage in autumn. It was believed that the summer decrease was due to the spring crop of ticks becoming engorged so that few or no ticks were left to infest sheep until these had moulted'. MacLeod objected: 'Were this so, the termination of the spring period of high infestation would depend to a large extent on the density of stocking, *heavily stocked ground becoming exhausted of its unfed tick population earlier than ground lightly stocked.*' MacLeod then gives incidence curves (1939, Fig. 6) for three flocks stocked at 1 sheep to 5 acres, 1 sheep to 2 acres and 5 sheep to 3 acres respectively. These curves all wane and end in company. MacLeod concludes that 'stocking density is not related to time of relapse of the incidence curve', and hence that the two-brood theory is untenable.

Weekly tick counts on sheep in spring show, broadly speaking, a weekly increase in infestation up to the peak, and a weekly decrease thereafter (see Milne, 1944). Since the host potential of a fixed flock does not change from week to week, a reasonable interpretation of this rise and fall of infestation is that *a varying number of ticks become active for the first time each week of the active season.* In this case, increasing the sheep stock could lower the level of individual infestation (as on Crag farm, see later), but it could not cause the season to end before the last week in which ticks became active. Thus the idea that increasing the sheep stock could test the two-brood theory is doubtful. Provided the sheep is the main host of the tick (as work in progress is indicating) a seemingly better method of testing the two-brood theory is by delayed stocking with sheep, i.e. withholding the main host until the normal active season is nearly at an end. If the theory is valid, then a tick-infested grazing, kept free of sheep and other domestic stock from the beginning until well past the peak of the normal active season, should show a prolongation of activity into the normal off-season. This has been tested three times on two farms, Hethpool and Crag.

2. METHODS

Crag. Adjoining the fell on this farm there is a deteriorated 'intake' (enclosed section of fell) now reverted for many years to its original state, i.e. intake and fell have the same type of vegetation cover. The intake is grazed along with the fell. Both have been tick-infested for very many years. Thus it might reasonably be supposed that they support similar population densities of ticks. This was shown to be true at the end of April 1941 when 10 sheep on the fell had an average of 25·6 female ticks while 10 sheep on the intake had 22·7 (the difference between these means being insignificant—see Milne, 1943). Five acres of the intake were enclosed (grazing D) on 11 March 1940. Thereafter no farm stock were allowed within the enclosure until 10 sheep, previously held in a tick-free pasture, were introduced on 6 May. Weekly counts on the 10 sheep were started on 13 May and were compared with similar counts made on the open fell (grazing N) which is constantly stocked at 1 sheep to 5 acres. Counts had to be discontinued on grazing D (the

enclosure) because at a stocking of 1 sheep to half an acre the pasture was insufficient after 10 June. The different stocking densities on grazings D and N were necessary (*a*) to check MacLeod's finding; (*b*) to ascertain whether heavier stocking results in a lower individual level of infestation in sheep; and (*c*) to give every unfed tick on grazing D the best chance possible of being picked up soon after becoming active—10 hill sheep confined to 5 acres of fell quarter the ground very thoroughly in grazing. Thus, apart from delay in stocking and differences in stocking density, grazings D and N were similar,

Hethpool. On this farm, two 200-acre adjacent hill grazings, D and N, identical as to vegetation cover and sheep-stocking density (1 sheep to 1½ acres) have been tick-infested for many years. As at Crag, it is therefore reasonable to assume similar ground populations of ticks. On 18 March 1940 and 6 April 1941, the sheep stock was completely withdrawn from grazing D. These sheep, meanwhile on an uninfested lowland pasture, were reintroduced to grazing D on 4 May 1940 and 6 May 1941; and weekly tick counting on 10 sheep commenced on 11 May 1940 and 17 May 1941. In 1940 the stocking of grazing D was at the normal density from the date of reintroduction onwards; in 1941 the stocking was half normal from 6 to 18 May, three-quarters from 19 to 23 May, and at normal density from 24 May onwards (this undesired complication was dictated by farming difficulties). Grazing N carried its normal sheep stock throughout the entire spring seasons, when routine counts on 10 sheep were made weekly. Unfortunately, because of farming difficulties, counts on grazing D had to be discontinued after 4 weeks in 1941. On grazing N, counts for the first part of the active season in 1941 are incomplete for the same reason, but the series of counts for the spring season of 1940 is complete. At Hethpool, the aim was to have conditions (with the exception of the delay factor alone) as nearly similar as possible on the D and N grazings.

The same age-class of sheep was used for counts in all cases; and on each grazing the same 10 sheep were used for each weekly count (there were only 10 sheep on Crag grazing D). After the reintroduction of sheep, at least 1 week was allowed for sheep to attain the current level of infestation on the D grazings before counting began.

3. DELAYED STOCKING WITH SHEEP AND THE ACTIVITY INCIDENCE CURVE

Clearly, in all three experiments (see Table 1), the D grazing had been deprived of sheep hosts (and other farm stock) for more than half of the spring-tick season, sheep not being introduced until the normal peak time had passed. On the other hand, the N grazings carried their normal sheep stock throughout the spring-tick season.

Fig. 1 and Table 1 show that in all three experiments the *trend* of the normal (N) and delayed (D) stocking curves is similar. Delay in stocking with the chief host, sheep, does not give rise to any appreciable prolongation of activity beyond the normal end of activity, at least not more than a few days. As defined in the introduction, the two-brood theory is therefore untenable as the *complete*

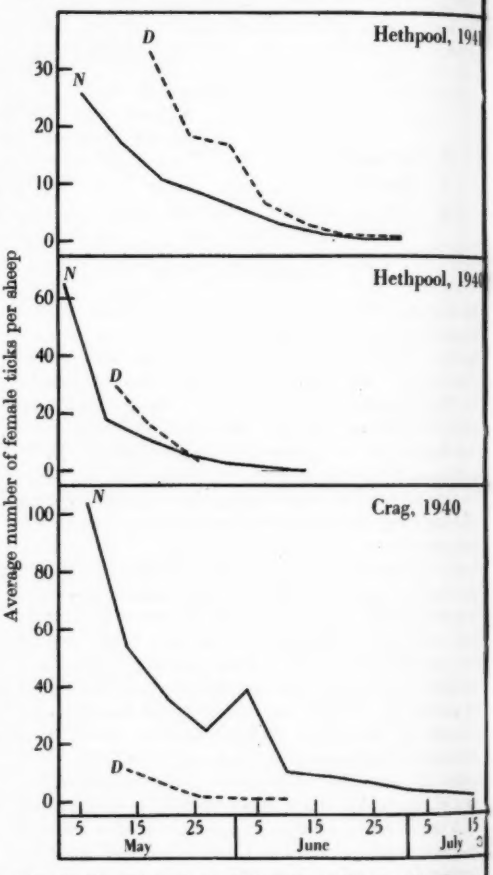

Fig. 1. Experiments in delayed stocking with sheep. Female tick activity curves on grazings D (stocking delayed) and N (stocking not delayed).

explanation of bimodal annual activity. At least some of the autumn ticks, however, should result from the spring-fed ticks. Hence the two-brood theory may contribute in some degree to the explanation.

Female ticks drop off after 7–11 days gorging (MacLeod, 1932); in the following, for simplicity in calculation, the gorging period is assumed to be 7 days.

At Crag 1940 the counts on grazing D were discontinued on 10 June (see Table 1). Let it be assumed that the weekly average per sheep is thereafter 0·4 ticks, i.e. the average for 10 June. (Considering the trend of the tails of the N and D curves for Crag in 1940 and Hethpool in 1941, this assumption is generous.) Then the 5-acre enclosure, grazing D, yielded 215 female ticks (total from the grazing D during the entire spring season have been fed under delayed stocking conditions.

At Hethpool 1940 tick counts ceased on grazing D on 25 May. If, as above, 4·4 ticks average per sheep per week is allowed thereafter, the total female ticks per 1½ acres (i.e. per 1 sheep) in counts from 11 May to 13 June was 63. The corresponding number on grazing N was 39 female ticks. During the entire

Table 1. *Average numbers of female* Ixodes ricinus *per sheep on grazings where stocking was delayed* (D) *and not delayed* (N)

Hethpool 1940				Hethpool 1941			
	200 acres: 1 sheep to 1·5 acres		200 acres: 1 sheep to 1·5 acres		200 acres: 1 sheep to 1·5 acres		200 acres: 1 sheep to 1·5 acres*
Date	N	Date	D	Date	N	Date	D
15 Mar.	1·6	18 Mar.	Sheep withdrawn	21 Apr.	14·3	6 Apr.	Sheep stock withdrawn
21	7·1	—	—	28	15·7	—	—
28	41·4	—	—	5 May	25·9	6 May	Sheep returned
4 Apr.	33·2	—	—	12	17·1	—	—
11	83·4	—	—	19	10·8	17 May	33·0 (S)
18	73·8	—	—	26	8·5	24	18·5 (S)
25	65·9	—	—	2 June	5·7	31	16·9 (S)
2 May	65·1	4 May	Sheep returned	9	3·0	6 June	6·6
9	18·4	11	29·5 (S)	16	1·1	14	2·6
16	11·0	17	15·6	23	0·4	20	0·9
23	5·7	25	4·4	30	0·3	30	0·6
30	2·7	Counts discontinued because of farming difficulties					
6 June	1·1						
13	0·2						

* On grazing D stocking was half normal, 6–18 May; three-quarters, 19–23 May; and normal thereafter.

Crag 1940					
	Open fell: 1 sheep to 5 acres	Enclosed fell (5 acres): 1 sheep to ½ acre		Open fell: 1 sheep to 5 acres	Enclosed fell (5 acres): 1 sheep to ½ acre
Date	N	D	Date	N	D
11 Mar.	0·9	Sheep withdrawn	20 May	35·7	5·5
18	2·7	—	27	24·2	1·9
25	10·6	—	3 June	38·3	0·7
1 Apr.	18·2	—	10	10·1	0·4
8	41·6	—	17	8·6	Counts discontinued because enclosure could no longer support sheep
15	48·0	—	24	6·2	
22	33·9	—	1 July	3·9	
29	90·1	—	8	2·8	
6 May	103·7	Sheep returned	15	1·7	
13	53·6	11·0			

(S), see text.

10 sheep) in counts from 13 May to 15 July. During the same period, 5 acres of fell, grazing N, yielded 185 female ticks (total from 1 sheep). Now 5 acres of grazing N, during the entire spring season—11 March to 15 July, yielded a total of 535 female ticks on the average. According to the two-brood theory a like total could be expected on grazing D before activity came to an end. Clearly, however, only about half the ticks actually available in spring season grazing N yielded 411 female ticks per 1½ acres. Hence about four-fifths of the ticks actually available during the spring season on grazing D went unfed because of delayed stocking conditions.

Bearing in mind that stocking was half normal on grazing D at Hethpool on 6 May 1941 and was not graded up to normal until 24 May, a proportion of the ticks on grazing D again went unfed due to

delayed stocking. Unfortunately the lack of counts for the first part of the spring season, and the various stockings on grazing D introduce too many complexities for a dependable calculation to be made.

It is safe, however, to conclude that at least half the female ticks actually available on a grazing during the entire spring season remain unfed if stocking with sheep is delayed until 0–3 weeks after the normal peak. The ticks which miss feeding must comprise the majority of those active before the sheep were introduced.

At Crag the average total yield of female ticks per sheep for all the counts on grazing D (13 May–10 June, see Table 1) was 19·5. The corresponding yield for grazing N is 161·9. Being ten times more heavily stocked than grazing N, the average infestation per sheep on grazing D might be expected to be 0·10 times that on grazing N, since every sheep will gather its share of the existing active tick population. Actually it is 0·12 (i.e. 19·5/161·9). Thus, as suggested in the introduction, an increase in stocking density lowers the average infestation per sheep.

4. DISCUSSION

After the reintroduction of sheep to grazing D, the corresponding average total yields of ticks per 5 acres (Crag) or 1·5 acres (Hethpool) on grazings N and D were:

	N	D
Crag, 13 May–10 June	161·9	195·0
Hethpool 1940, 2–25 May	35·1	49·5
Hethpool 1941, 31 May–30 June*	10·5	27·6

* The first two counts on grazing D are omitted because of complex stocking inequalities—see Table 1.

The yields are higher for grazing D in all three cases. The two lots of 10 sheep used for tick-counting in each experiment were randomly chosen from groups in which avoidable sources of group variation had been eliminated (see Milne, 1943, p. 243, iv). Hence the possibility of the D sheep having greater susceptibility to infestation can be ruled out as too great a coincidence. The consistently higher yields could arise from grazing D having the higher ground population of ticks. Experiments (unpublished) have shown, however, that neighbouring stretches of land greater than 1 acre

(i) having similar vegetation cover;

(ii) habitually grazed by the same breed of sheep at the same stocking density; and

(iii) tick-infested for the same length of time,

also have similar tick population densities. Grazings D and N at Hethpool meet these requirements; so also did grazings D and N at Crag before the experiment. In the latter case counts did show similar population densities (see Methods, and last paragraph, section iii). The consistently higher total yields on the D grazings therefore suggest that slightly more ticks are active after delay in stocking than would be without delay. The small excess must be a residue from the ticks active earlier when no host was available. Since this residue comes nowhere near to accounting for all these ticks, individual female ticks must, on the average, be active for a period shorter than half the spring season. Comparison of weekly averages gives a rough indication how much shorter:

Hethpool data. In Table 1 (S) indicates that the difference between D and N means is significant (statistically—see Milne, 1943). The infestation level on grazing D is significantly higher than the corresponding level on grazing N for about the first 7–14 days in 1940 and the first 21–28 days in 1941. (The longer period in 1941 is probably due to the subnormal stocking for the first 17 days—see Table 1.) This does not mean that the excess of D over N at any time later is necessarily insignificant; counts on 10 sheep demand rather large differences to prove significance. Indeed, the consistency in all three experiments indicates that residual effect of delayed stocking is evident for several weeks, but at a quickly diminishing rate (see Fig. 1). In 1940 only the first two D counts are higher, $29·5 + 15·6 = 45·1$. Taking the corresponding two N counts and adding the previous count the result is $65·1 + 18·4 + 11·0 = 94·5$, which is significantly higher than 45·1. Thus not even all the ticks active in the week preceding reintroduction of sheep were active after the succeeding fortnight. Stocking complexities render a similar calculation for 1941 difficult and doubtful.

Crag data. The enclosure, grazing D, was grossly over-stocked to compel more thorough quartering of the ground by sheep in search of food. Thus any active tick would not have long to wait for a host. If the means for D be multiplied by 10, the resultant values represent ticks per 5 acres. These latter may be compared with the means for N also representing ticks per 5 acres, since the fell was stocked at 1 sheep to 5 acres:

N	53·6	35·7	24·2	38·3	10·1	Total 161·9
D	110	55	19	7	4	,, 195·0
	(S)	—	—	(S)	(S)	

D is significantly higher for the first count alone and is less on and after the third count, significantly so in the last two. Now for D, $110 + 55 = 165$; while for N, after adding the two previous counts, $90·1 + 103·7 + 53·6 + 35·7 = 283·1$, which is barely significantly greater than 165. But since between the second and third counts the curve for D passes below that for N, the total of 165 for D must have contained a proportion of individuals normally belonging to the last three counts for D. This would arise from the gross over-stocking on D causing

some ticks to be picked up earlier than they would be at the normal lower stocking. Thus, not even all the ticks active in the fortnight preceding reintroduction of sheep at Crag D were active after the succeeding fortnight.

The conclusions are therefore:

(i) Delay in stocking results in a slightly higher-than-normal population of active ticks, which is fairly quickly absorbed after reintroduction of hosts.

(ii) A varying proportion of the tick population becomes active each week throughout the tick season, increasing up to the peak and decreasing thereafter.

(iii) The *average* duration of individual activity in a natural population of female *I. ricinus* is probably not more than 1 month, perhaps less. Some individuals may, of course, be active for longer periods. Duration of activity will vary with the current meteorological conditions. It probably varies inversely with age, also. This idea finds some support in the behaviour of *Dermacentor andersoni* Stiles, a three-host tick not unlike *I. ricinus* in its habits. In Western Montana, Philip (1937) flagged the same strips of land bi-weekly or weekly for 6 years, always returning the ticks to their own small piece of ground. He states 'ticks marked early in each season were never taken in any great proportion during subsequent collections', but records showed 'activity throughout the adult tick season on the part of some individuals. On the other hand, as many as 11 ticks marked at the beginning of 1930 were taken on 17 April a year later, with a noticeable decline of such ticks by 5 May without a concomitant decrease of those marked earlier the same season (1931). This would possibly indicate early estivation or death of some older individuals not finding hosts'.

5. PRACTICAL CONCLUSIONS

The most striking fact about the experiments is that delay in stocking with the chief host does not prolong the activity of a female tick population to any marked degree. The practical application of this finding is that farm stock can have the run of infested land in the normal off-seasons of activity without danger of infestation even if the land has not been grazed in the seasons of activity. Tick populations could be considerably reduced by grazing only during the offseasons, since farm stock, especially sheep, have by far the highest host potential of all, and a full blood meal is required by the female tick before she can lay eggs. How far this can be applied to hill-farming is chiefly a matter of economics, but the idea is used by Cumberland farmers on a restricted scale as follows: Lambs are most susceptible to tick troubles in their early days. Many Cumberland men who lamb on their relatively low-infested inbye land delay putting the flock back on the highly infested fell until spring activity is well on the wane. Thus lambs are well grown before they come into contact with ticks during the medium autumn infestations.

6. SUMMARY

In three experiments, the chief hosts, sheep, were withheld from a grazing until 0–3 weeks after the normal spring peak of female tick activity. This did not give rise to any appreciable prolongation of female tick activity into the normal summer off-season. More than half the female ticks actually available during the entire spring season were unfed when activity came to an end. This was due to the withholding of hosts. Hence that aspect of the 'two-brood' theory which claims that activity ends in spring, because all the available ticks are fed, is untenable as the complete explanation of bimodal annual activity.

Increase in sheep-stocking density, over the normal for a grazing, lowers the average infestation per sheep for that season at least.

The experimental data suggests the following:

(i) Delay in stocking a grazing with sheep results in a slightly higher-than-normal population of active female ticks which is fairly quickly absorbed after reintroduction of sheep.

(ii) A varying proportion of the tick population becomes active each week throughout the tick season, increasing up to the peak and decreasing thereafter.

(iii) The *average* duration of individual activity in a natural population of female ticks is probably not more than 1 month, perhaps less.

Practical applications of the findings to hill sheep farming are indicated.

Thanks are due to Dr V. B. Wigglesworth, F.R.S., Director, Agricultural Research Council Unit of Insect Physiology, and Dr A. E. Cameron, Edinburgh University. Dr Cameron suggested the delayed stocking approach. The funds for the work were provided by the Agricultural Research Council, and the Duke's Memorial Fund (per Mr W. Lyle Stewart, M.R.C.V.S.).

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THE EFFECTS OF ACIDS ON A SOIL NEMATODE

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(With 3 Figures in the Text)

INTRODUCTION

The effects of acids on nematodes have been studied mainly upon various species of *Ascaris* (see Weinland, 1904; Flury, 1912; Slater, 1928; von Brand, 1934) and upon the vinegar eelworm *Turbatrix aceti* (Müller) (see Bělehrádek & Schwarz, 1928; Stoklasa, 1933).

As these worms are very specialized in their habitats, it was thought desirable to undertake a similar study upon a more typical member of the phylum, *Rhabditis terrestris* Stephenson, to see to what extent peculiar behaviour towards acids was general throughout the group. Among other things, the toxicity of the different acids was investigated, since it was thought that any basic principles relating toxicity to the physical and chemical properties of the molecule might be of theoretical and practical interest in the development of new anthelmintics.

METHODS

The following acids were used, each being made up in glass distilled water: hydrochloric, nitric, sulphuric, formic, acetic, propionic, butyric, isovaleric, glycollic, lactic, oxalic, malonic, succinic, malic, tartaric, and citric. The purest available grade of B.D.H. chemicals was employed in each case.

The general method of study was identical with that previously given (Stephenson, 1942*b*; 1943). The times required to kill the worms in the various solutions were noted, an individual being considered dead when no movement was observed during 4 min. of continuous observation. When half of each batch of ten worms had satisfied this condition, the time of cessation of movement was noted to the nearest $\frac{1}{2}$ min.

The *p*H values of certain solutions which are given below were obtained by calculation, not by measurement. In the case of the monobasic acids they were calculated from the formula given by Michaelis (1926, p. 40): $[H^+]=\sqrt{(\frac{1}{4}K^2-KA)}-\frac{1}{2}K$, where K is the dissociation constant, and A the concentration of the acid in gram-molecules per litre. The values for the dibasic acids can be calculated from a similar formula, the derivation of which is shown in the Appendix:

$$x^3+K_1x^2+(2K_1K_2-K_1A)\,x-2K_1K_2A=0.$$

Here x is the concentration of hydrogen ions, A the concentration of acid (in gram-ions and gram-molecules per litre respectively), and K_1 and K_2 the dissociation constants for the first and second stages of dissociation. The values of the dissociation constants were taken from the tables of constants, and the cubic equations were solved by the method of successive approximations. The final *p*H values are given to the nearest 0·05 *p*H units.

RESULTS

Qualitative effects

(*a*) *On movement.* In all solutions abnormalities in movement occurred. Minor differences were noted in the initial effects of the different acids, particularly in the more dilute solutions, and various descriptive terms such as 'jerky twitching', 'lashing', and 'spiralling', convey a general impression of these effects. In the final stages there is a slow 'jerky' movement which decreases and finally ceases. After this no recovery ever occurs even on transference to tap water, and it was therefore concluded that cessation of movement coincided with death. After death in most of the acids, the body of the animal slowly straightens out.

(*b*) *On the cuticle.* In solutions of *p*H 0·5–3·5, the cuticle of the worms becomes very sticky which causes the animals to adhere to one another, to the containing vessel, and to the fine glass needles sometimes used for separating them. This stickiness of the cuticle preceeds death, and usually disappears about the time of death. With hydrochloric acid and most of the dibasic acids, just before death, large clear bulges occurred on the lateral surfaces of about 40 % of the specimens examined. These bulges (see Fig. 1) contained a clear jelly and were believed to be due to a swelling of the internal layers of the cuticle.

(*c*) *Other effects.* A variety of distortions of the body occurred in different acids while the animals were dying. These included a darkening of the protoplasm in the anterior part of the body, a slight twisting of the oesophagus, and pushing of the second oesophageal bulb into the anterior part of the intestine. Certain of these changes are shown in Fig. 1. After death the whole body gradually

swelled, its length in particular showing noticeable increases which coincided with the straightening out of the body.

Quantitative effects

The quantitative effects of the different acids can be compared most readily by measurements of the times required to kill the worms.

Preliminary experiments were performed using formic, acetic, propionic, and butyric acids in order to ascertain the relationship between the strength

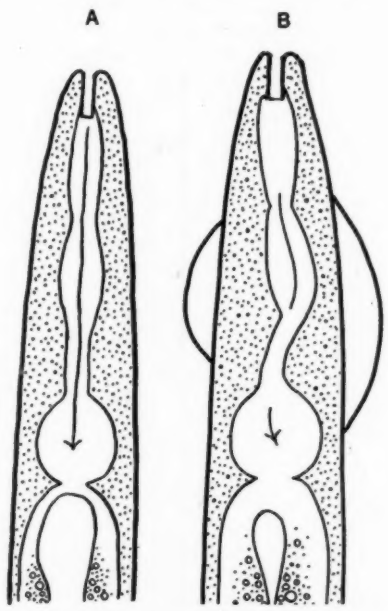

Fig. 1. A. The normal appearance of the anterior of the body. B. Appearance after immersion in certain acid solutions. Note the lateral swellings in the cuticle, the twisting of the oesophagus, and the swelling of the anterior part of the intestine.

of each acid and its effect on movement. The results are shown in Fig. 2 where the logarithms of the survival times in minutes are plotted against the logarithms of the normalities of the acids. The graphs obtained approximate closely to straight lines, and the two variables can thus be connected by formulae of the type $T = A/N^b$, where T is the survival time, N the normality of the acid, and A and b are constants whose values depend on the nature of the acids and the units employed. This formula can be alternatively expressed:

$$\log T = -b \log N + k,$$

where k is $\log A$. Bělehrádek & Schwarz (1928) obtained a similar expression for the effect of different concentrations of acids on the vinegar eelworm and upon *Tubifex rivulorum*. Their data show that the survival times of *Tubifex* and *Rhabditis* in a given range of acid concentrations are approximately the same, but that solutions about 20 times stronger are required for the vinegar eelworm.

The most probable values of the constants b and k in the above expression were calculated by the method described in Snedecor (1938) and are given in Table 1. Undue stress should not be laid upon these results, since they were derived from preliminary experiments especially as it was difficult to obtain consistent values for b and k in experiments undertaken at different times. Various trials with the three mineral acids and acetic acid all gave straight line graphs when the logarithms of survival time are plotted against the logarithms of the normality of the acid, but different trials gave different straight lines. The effect of the following variables on this lack of uniformity was investigated: (*a*) variations

Table 1

Acid	b	k
Formic	0·93	−0·25
Acetic	1·00	0·32
Propionic	0·83	−0·23
Butyric	0·94	0

in the temperature of culturing (from 20 to 23° C.), (*b*) doubling the population density used to start the cultures, and (*c*) increasing the age of the cultures by 2 days, i.e. selecting the later maturing individuals. In each case care was taken to use only females which were about to begin laying eggs, and as an additional precaution, all these experiments were carried out at an approximately constant temperature (25 ± 1° C.). The results obtained showed that the main effect was due to increasing age of the cultures. In the course of 2 days there was an average change of 0·05 in the value of b, and an average change of 0·23 in the value of k. These changes were highly significant from a statistical point of view, as shown by the 't' test of Snedecor (1938). Expressed as percentages of their absolute values, the changes in the course of 2 days were: for b, 7 %; for k, 43 %. Since care had been taken to select individuals at the same stage of development, the individuals had apparently altered in some unknown way when the age of the culture was increased. It is impossible to obtain consistent values of b and k because, on a proportional basis, changes of 1·5 and 9 % respectively, are to be expected in the course of a 10 hr. experiment. Hence further attempts to obtain mathematical relationships between the normalities and survival times were severely restricted.

In view of this, to obtain accurate comparisons between the effects of different acids, it was necessary to perform a series of experiments with as many acids as possible in the shortest time, and the number of determinations on each was therefore reduced to one. Equal normalities (0·206) of each acid were used because if solutions of the same *p*H had been employed, extremely low concentrations of the mineral acids would have been required, and moreover these low concentrations would have been difficult to maintain in contact with glassware. To minimize the effects of increasing age of the animals during the course of an experiment, the acids were employed in a definite order, and the experiments repeated as soon as possible, with the animals from the same culture, using the acids in the reverse order. Each determination was repeated ten times, using animals from five cultures. The mean survival times and their standard errors are given in Table 2.

It should be noted that the mineral acids are more toxic than the organic acids, and this is accompanied by lower *p*H values. On the other hand, the lack of correlation among the organic acids between the toxicity and the *p*H values suggests that this is not the only factor involved. Besides differing in hydrogen-ion concentrations, the solutions differ in the nature and concentration, both of the anions and of the undissociated molecules. Additional data were therefore required to assess the importance of each of these factors.

If the organic acids are made up in a fairly low concentration of mineral acid, the high hydrogen-ion concentration of the latter results in the former being almost completely undissociated. In the experiments which follow, a given volume of 0·206 *N* organic acid was mixed with an equal volume of 0·0206 *N* HCl. The *p*H differences between the solutions are now much reduced, and the concentrations of undissociated acids almost identical. Succinic, tartaric, and citric acids were not used in these experiments because they deteriorate in the course of a few days owing to bacterial action. The mean survival times in the remaining mixtures are given in Table 3. In these experiments the differences between the toxicities of the acids must be mainly due to the different effects of the undissociated acids. Tables 2 and 3 show that the relative toxicities of the acids are not materially altered. The main differences between ordinary aqueous solutions of the acids (Table 2) are therefore due to the undissociated molecules.

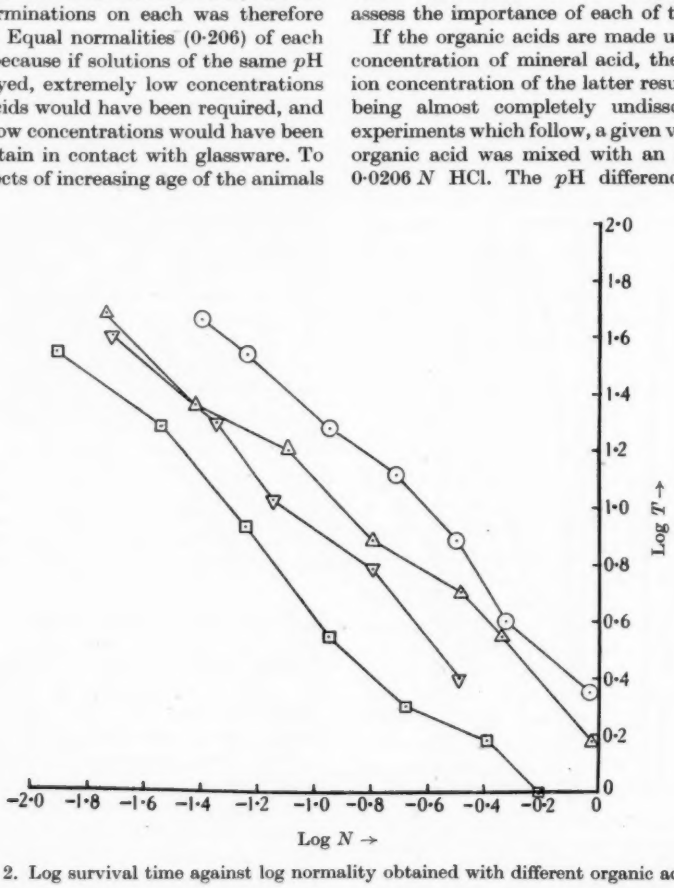

Fig. 2. Log survival time against log normality obtained with different organic acids.

Acetic ⊙—⊙—⊙—⊙; Propionic △—△—△—△; Butyric ▽—▽—▽—▽; Formic ⊡—⊡—⊡—⊡.

In order to assess the effect of changes in hydrogen-ion concentration, different strengths of HCl were employed, because previous work (Stephenson, 1943) had shown that at the concentrations employed, chloride ion 'alone' has only minor effects on the movement of the worm. Of the acids shown in Table 3, omitting oxalic acid on account of its large dissociation constant, formic was the most, and malic the least toxic. These acids were therefore used as characteristic organic acids. They were made up in 0·01 *N* HCl, their solutions thus being almost entirely undissociated, and at about the same *p*H (see Table 4). In determining the toxicity of the undissociated acids, allowance was made for the toxicity due to the HCl present. In making this allowance it was assumed that the reciprocal of the survival time in any solution is a measure of its toxicity. Thus, in a mixture of HCl with organic acid, the reciprocal for the HCl alone was subtracted from the reciprocal for the mixture, and the difference taken as a measure of the toxicity due to the organic acid alone. It will be noted that this involves assumptions regarding the toxicity of mixtures, which, while theoretically debatable, enable a simpler presentation of comparative effects than would otherwise be the case.

Tables 2 and 3. *Mean survival times in minutes (and their standard errors) on immersion in* 0·206 *N solutions of the acids shown (Table* 2*); and in mixtures of equal volumes of* 0·0206 *N* HCl *and* 0·206 *N solutions of organic acids (Table* 3*). In each table the acids are arranged in order of toxicity, and the p*H *values calculated, to the nearest* 0·05

Table 2

Acid	Mean survival time in min.	Standard error of mean	Calculated *p*H
Nitric	1·26	0·07	0·70
Hydrochloric	2·06	0·06	0·70
Formic	2·57	0·23	2·20
Oxalic	2·67	0·20	1·30
Sulphuric	3·10	0·17	1·05
Isovaleric	3·80	0·25	2·80
Butyric	6·03	0·31	2·80
Malonic	7·56	0·44	1·90
Acetic	8·31	0·55	2·80
Glycollic	8·38	0·58	2·25
Propionic	8·94	0·55	2·80
Lactic	9·40	0·51	2·30
Malic	9·62	0·55	2·35
Tartaric	11·49	0·74	2·00
Citric	14·54	0·67	?
Succinic	16·77	0·50	2·60

Table 3

Acid	Mean survival time in min.	Standard error of mean	Calculated *p*H
Oxalic	3·52	0·14	1·35
Formic	3·93	0·38	1·90
Isovaleric	6·65	0·60	1·95
Butyric	7·61	0·75	1·95
Propionic	8·32	0·75	1·95
Glycollic	8·33	0·64	1·95
Malonic	8·41	0·34	1·80
Lactic	8·57	0·75	1·95
Acetic	8·76	0·81	1·95
Malic	10·53	0·56	1·90

In Table 4 values are given for the toxicities of different normalities of HCl, undissociated formic acid, and undissociated malic acid, and the *p*H values of the solutions are also shown. In Fig. 3 the toxicities are plotted against normalities, and the figure shows that, over the range investigated toxicity, as defined above, is approximately proportional to normality. Comparison of the slopes shows that a given increase in the concentration of HCl produces about 2·3 times the effect of an equal increase in concentration of undissociated formic acid, and about 17 times the effect of an equal increase in concentration of undissociated malic acid. If absolute constancy of hydrogen-ion concentration could have been maintained in the organic acid solutions, these ratios would have been slightly larger. It is evident that differences in *p*H are of major importance.

Table 4. *The toxicities and p*H *values of different normalities of* HCl, *and of different normalities of formic and malic acid containing* 0·01 *N* HCl. *In calculating the toxicities of the latter, the value for the toxicity of* 0·01 *N* HCl *was subtracted*

Acid	Normality	Toxicity (reciprocal of survival time in min.)	Calculated *p*H
HCl	0·01	0·069	2·00
	0·05	0·144	1·30
	0·09	0·252	1·05
	0·13	0·348	0·90
	0·17	0·416	0·80
Formic acid of the normalities shown, in 0·01 *N* HCl	0·04	0·067	2·00
	0·08	0·086	1·95
	0·12	0·131	1·95
	0·16	0·165	1·90
Malic acid of the normalities shown, in 0·01 *N* HCl	0·04	0·009	2·00
	0·08	0·014	1·95
	0·12	0·021	1·95
	0·16	0·025	1·90

DISCUSSION

(*a*) *General effects.* The stickiness of the cuticle which sometimes occurs in acid solutions is evidently due to a change in the physical properties of the surface layer of the cuticle such as might be

expected when the protein constituents at the surface have reached the end of the isoelectric zone. If this is so, the isoelectric 'point' is probably at about *p*H 3·5. Under certain conditions, not always identical with those above, bulges form in the cuticle at the anterior region of the body. These may be due to the swelling of the proteins comprising the middle layers of the cuticle, similar to the swelling of gelatin and other proteins in acid media (Lloyd & Shore, 1938). The bulges were only seen at the anterior part of the body, which suggests that, in this region there is a specialized portion of the cuticle which is more susceptible to the action of the acids than is the remainder.

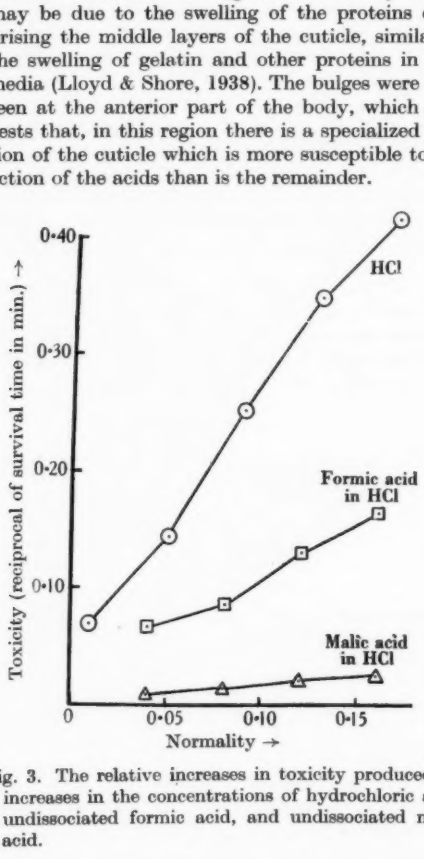

Fig. 3. The relative increases in toxicity produced by increases in the concentrations of hydrochloric acid, undissociated formic acid, and undissociated malic acid.

The post-mortem swelling of the worms is probably due to a similar swelling of proteins, and is of interest from the systematic point of view because it suggests that fixation in acid media will tend to increase dimensions. Since dimensions are of great importance in nematode systematics, it would seem desirable to standardize fixation methods throughout the phylum to avoid complications of this type.

(*b*) *Mineral acids.* The toxicity of a substance depends upon its rate of penetration and upon other factors. Thus the penetration of small quantities of highly poisonous substances produces greater effects than those of large quantities of innocuous substances. Since the importance of these other factors can rarely be assessed, explanations in terms of rates of penetration are generally adhered to below.

Table 2 shows that strong mineral acids produce greater effects than do the organic acids of equal normalities. The results given in Table 4 show the effect of changes in hydrochloric acid concentration compared with changes in concentration of certain undissociated organic acids, and, while the interpretation of the results is not free from objections, they do emphasize the importance of the mineral acids. This is contrary to the general impression that weak organic acids are much more active physiologically than the stronger acids. This impression has largely arisen from work by Osterhout (1925), Osterhout & Dorcas (1925), and Jacques (1936), in which it was shown that weak acids such as H_2S and carbonic acid penetrate the cell much more readily in the form of undissociated acids, than they do as ions. In addition, work upon the relative merits of different acids as activators of artificial parthenogenesis has shown that in the case of sea urchin eggs (Loeb, 1913) and starfish eggs (Lillie, 1927), the monobasic fatty acids are much more effective than the mineral acids.

In the case of *Rhabditis*, at a given normality (0·206), the strong acids are much more effective than the weak ones. Essentially similar results have been obtained on the tissues of Mollusca and Holothuria, and with intact earthworms by Crozier (1916), with testes of holothurians by Harvey (1914), and in mammalian taste experiments (Paul, 1922; Taylor, 1928). Crozier's impression (1916) that the comparatively greater effects of the weak acids in the case of echinoderm eggs is an exception rather than the rule, is supported by most of the later work, including the results of the present paper. It will be noted that the relative toxicities of the mineral acids agree with the well-known lyotropic series (see Davson & Danielli, 1943, pp. 159–63), so that while the hydrogen ions are extremely important, a second factor, presumably due to the effects of the anions on colloids, evidently operates.

(*c*) *Organic acids.* In Table 2 the different effects of the dissociated organic acid are compared. Certain of these differences must be due to different *p*H values. Tables 2 and 3 show that the relative toxicities of dissociated and undissociated acids are very similar. Therefore it is concluded that the main differences between the effects of the acids are due to the undissociated molecules. In attempting to explain these differences the following possibilities are considered: (i) molecular size, (ii) polarity of the molecule, and (iii) specific groups in the molecule.

(i) *Molecular size.* Certain of the above differences might be explained on the basis of a sieve theory, as in the case of lobster cuticle (Yonge, 1936), and bacteria (Marklund, 1936; Ruhland & Hoffmann,

1925). Thus the larger molecules (malic, tartaric, citric, and succinic acids) all have a comparatively slight action. Against this, it should be remembered that, since equinormal solutions were used, only one-half or one-third the molecular strength as compared with a monobasic acid would be present in these cases.

(ii) *Polarity of the molecule.* Explanations in terms of molecular size do not hold in the case of the fatty acid series, where increasing the length of the $-CH_2-$ chain generally results in an increase in toxicity. Thus omitting formic acid: acetic = propionic < butyric < isovaleric. This can be interpreted on the basis of the alternative theories of adsorption or of increased lipoid solubility. The relationship between the concentration of a given acid and its effect on the animal (see Fig. 2) is a further support for a theory dependent upon the polarity of the molecule, since it agrees with relationships which are typical of adsorption processes (see McBain, 1932). It is not within the scope of the present paper to decide between the adsorption and lipoid solubility theories (see Davson & Danielli, 1943).

(iii) *Specific groups in the molecule.* Crozier (1916) showed that, in the case of the sea slug, *Chromodoris zebra*, when a hydrogen atom was replaced by a hydroxyl group, a greater rate of penetration occurred (e.g. lactic > propionic; tartaric > malic > succinic; salicyclic > benzoic). Taylor (1928) drew the same conclusion from his experiments on mammalian taste. This was not observed in the case of *Rhabditis*.

None of the above theories explain the case of formic acid, which is much more toxic than the remainder of the fatty acid series. No adequate explanation of this can as yet be given. While this is also true of *Turbatrix aceti* (Bělehrádek & Schwarz, 1928), it does not apparently reflect any physiological peculiarity of the nematodes, since it applies equally to the mantle of *Chromodoris* (Crozier, 1916), the testes of *Stichopus* (Harvey, 1914), and the eggs of *Asterias* (Lillie, 1927). The ability of the vinegar eelworm to live in its peculiar environment is thus probably an extreme manifestation of a tolerance or resistance which is reasonably widespread throughout the animal kingdom, rather than a special feature of the nematodes.

SUMMARY

1. The effects of the following acids were studied: hydrochloric, nitric, sulphuric, formic, acetic, propionic, butyric, isovaleric, glycollic, lactic, oxalic, malonic, succinic, malic, tartaric, and citric.

2. The general effects of immersion in acid media include an increased stickiness of the cuticle in certain solutions, and the development of lateral bulges in the cuticle at the anterior region of the body, not necessarily in the same solutions. Movement gradually decreases, and death eventually occurs, often followed by swelling.

3. The survival times in various solutions were noted. Preliminary experiments showed that the logarithm of the survival time was approximately proportional to the logarithm of the normality of the acid. Later results showed great variability, and it was necessary to restrict the work to a single normality of each acid.

4. The toxicities of 0·206 *N* solutions of the sixteen acids were measured. These results showed that the mineral acids had markedly greater effects than the organic acids, and the importance of the former was further illustrated by using mixtures of HCl containing varying amounts of organic acids. The differences between the mineral acids may be explained by the lyotropic effects of the anions.

5. Equal normalities of organic acids were made up in a solution of HCl sufficiently strong to render the organic acids almost entirely undissociated. The relative toxicities of the mixtures were essentially the same as those of the dissociated acids.

6. The differences between the acids are thus partly due to the *p*H differences, and partly due to the dissimilar effects of the undissociated acids. The latter are believed to be due to a variety of causes, including molecular size, polarity of the molecule, and other factors as yet undecided.

I am deeply indebted to Prof. A. D. Hobson for advice and assistance throughout, and also to Prof. C. M. Yonge for help in the concluding stages. Thanks are also due to King's College, University of Durham, for financial assistance in the form of a post graduate studentship.

APPENDIX

Calculation of the *p*H of a solution of a dibasic acid:

$$H_2Y \rightleftharpoons H^{\cdot} + HY', \qquad HY' \rightleftharpoons H^{\cdot} + Y''$$

$$\frac{(H^{\cdot})\,(HY')}{(H_2Y)} = K_1, \tag{1}$$

$$\frac{(H^{\cdot})\,(Y'')}{(HY')} = K_2. \tag{2}$$

Let A be the concentration of acid,
p be the amount dissociating,
x be $(H^{\cdot})$ at equilibrium,
q be (HY') at equilibrium,
r be (Y'') at equilibrium,
} in gram-molecules or gram-ions per litre.

At equilibrium, the amount of Y which is no longer in the form of undissociated molecules of H_2Y will be in the form of HY' and Y''. Then the total amount of Y dissociating from H_2Y equals the sum of the concentrations of HY' and Y'', i.e.

$$p = q + r. \tag{3}$$

Similarly, the total amount of H dissociating from H_2Y equals the sum of the concentrations of H and HY, i.e.

$$2p = x + q. \quad (4)$$

From (1) $$K_1(A-p) = xq. \quad (5)$$

From (2) $$K_2q = xr. \quad (6)$$

Solving (3), (4), (5), and (6) for x:

$$x^3 + K_1x^2 + (2K_1K_2 - K_1A)\,x - 2K_1K_2A = 0.$$

Whence x, the hydrogen-ion concentration.

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A RECORD OF PARASITIC WORMS FROM FISHES IN ROCK POOLS AT ABERYSTWYTH

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Various records have, from time to time, been made of parasitic worms from marine fishes around the British Isles. Nicoll (1915) has compiled a very valuable list of the Trematoda, which class has received more attention than have the other parasitic worms of fishes. They have been recorded from Scottish waters by T. Scott (1901), from the Irish Sea by A. Scott (1904), from the Northumberland coast by Lebour (1908) and from Ireland by Bellingham in 1844 and Southern in 1912. The Cestoda have received less attention. Woodland (1927) has recorded some species from Plymouth, and a record of the trematode and cestode parasites of fishes from the Porcupine Bank, Irish Atlantic Slope, and Irish Sea has been made by Rees & Llewellyn (1941). The records of Nematoda and Acanthocephala are very scanty. Baylis & Jones (1933) include them together with trematodes and cestodes in a list of parasitic worms obtained from marine fishes at Plymouth.

The parasites of fishes from the rock pools on the shore at Aberystwyth have not been included in previous surveys and so the present paper is devoted to them. Over 100 fishes were dissected, including representatives of four species, *Onos mustelus* (L.), *Cottus bubalis* Euphrasen, *Crenilabrus melops* (L.) and *Blennius pholis* L. Specimens were obtained from rock pools on the shore about six miles north of Aberystwyth over a period of four months, from May to August 1942. The writer is indebted to Dr G. W. G. Maclennan for the collection of the material. The names used for the host fishes are those which appear in the British Museum *List of British Vertebrates*. On removal from the host the trematodes and cestodes were fixed in Gilson's fixative, *in toto* stained preparations being subsequently made for identification. The nematodes were fixed in hot 70% alcohol and examined in lactophenol, and the Acanthocephala, after evagination of the proboscis by pressure, were fixed in warm 70% alcohol and mounted unstained.

Seven species of trematodes, three of cestodes, two of nematodes and two of Acanthocephala were found. They were distributed as indicated in Table 1. No new species are recorded, but hosts believed to be new for any particular parasite are indicated by an asterisk (*). No monogenetic trematodes were found, although the gills of each host were carefully examined.

From the four species of host fishes examined fourteen species of parasitic worms have been recovered. There are eleven new host records, while six of the worms have been recorded previously from the same host species by other writers.

Identification of the trematodes, cestodes and nematodes presented no difficulty. The identification of *Leptorhynchoides thecatus* is perhaps open to question as hitherto it has been recorded by Van Cleave (1924) as being one of the commonest and most widely distributed of the parasites of fresh water fishes of northeastern North America. The species was present in the Leidy Collection examined by Van Cleave (1924), having occurred in the rock-bass (*Ambloplites rupestris*) from Lake George, New York. *Echinorhynchus oricola* von Linst., now regarded as a synonym of *Leptorhynchoides thecatus*, was recorded by v. Linstow in 1901 as having been found by Brandt in the mouth cavity of *Grystes* salmoides (= *Micropterus salmoides*) from New York. In the present instance a species closely resembling *Leptorhynchoides thecatus* has been found in the intestine of *Cottus bubalis* and *Onos mustelus*. The specimens in question have the diagnostic character of the species, namely, the cuticular collars around the proboscis hooks; the number and arrangement of the hooks too are similar. It is interesting to note that Baylis & Jones (1933) record *Echinorhynchus leidyi* from *Onos mustelus* at Plymouth. This species also, as they remark, had previously been recorded only from lake trout in the Great Lakes of North America.

The occurrence of the nematode *Ascarophis morrhuae* is of interest, as it is a little-known form. It was recorded by Baylis and Jones (1933) from *Trigla lineata* at Plymouth and a redescription of the female was given by Baylis (1933). In Table 2 the parasites are arranged according to their hosts.

Table 1

Parasite	Host	
TREMATODA		
Order Digenea		
Fam. Allocreadiidae		
Podocotyle atomon (Rud., 1802)	*Cottus bubalis*,	Intestine
	Onos mustelus	,,
	**Crenilabrus melops*	,,
Podocotyle reflexa (Creplin, 1825)	*Onos mustelus*	,,
Peracreadium genu (Rud., 1819)	**Onos mustelus*	,,
Lebouria alacris (Looss, 1901)	*Crenilabrus melops*	,,
Helicometra pulchella (Rud., 1819)	*Blennius pholis*	,,
Helicometra fasciata (Rud., 1819)	**Cottus bubalis*	,,
Stephanochasmus rhombispinosus Lebour, 1908	**Onos mustelus*	,,
CESTODA		
Order Pseudophyllidea		
Fam. Ptychobothriidae		
Bothriocephalus scorpii (Müller, 1776)	*Cottus bubalis*,	Intestine
	**Onos mustelus*	,,
Order Tetrarhynchidea		
Fam. Eutetrarhynchidae		
Grillotia erinaceus (v. Ben., 1858 (larva))	**Cottus bubalis*,	Stomach wall
Order Tetraphyllidea		
Scolex pleuronectis Müller, 1788	*Cottus bubalis*,	Intestine
	**Onos mustelus*	,,
NEMATODA		
Order Ascaroidea		
Fam. Ascaridae		
Contracaecum clavatum (Rud., 1809)	*Cottus bubalis*,	Intestine
	**Onos mustelus*	,,
Order Filarioidea		
Fam. Incert.		
Ascarophis morrhuae v. Ben., 1871	**Onos mustelus*	,,
ACANTHOCEPHALA		
Fam. Echinorhynchidae		
Echinorhynchus gadi Zoega, in Müller, 1776	*Onos mustelus*,	Intestine
Leptorhynchoides thecatus (Linton, 1891)	**Cottus bubalis*	,,
	**Onos mustelus*	,,

Table 2

Host	No. examined	Parasite	No. of fishes infested
Cottus bubalis	31	*Podocotyle atomon*	2
		**Helicometra fasciata*	1
		Bothriocephalus scorpii	11
		Scolex pleuronectis	7
		**Grillotia erinacea*, larva	1
		Contracaecum clavatum	2
		**Leptorhynchoides thecatus*	5
Onos mustelus	42	*Podocotyle atomon*	1
		Podocotyle reflexa	1
		**Peracreadium genu*	1
		**Stephanochasmus rhombispinosus*	1
		**Bothriocephalus scorpii*	3
		**Scolex pleuronectis*	3
		**Contracaecum clavatum*	2
		**Ascarophis morrhuae*	1
		Echinorhynchus gadi	7
		**Leptorhynchoides thecatus*	3
Crenilabrus melops	37	**Podocotyle atomon*	1
		Lebouria alacris	1
Blennius pholis	7	*Helicometra pulchella*	1

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CHEMOTROPIC STUDIES ON THE BLOW-FLIES *LUCILIA SERICATA* (Mg.) AND *LUCILIA CAESAR* (L.)

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CONTENTS

	PAGE
Introduction	168
1. Chemical examination of attractants:	
(*a*) Experimental procedure	169
(*b*) Results of analysis	169
2. Field trials on sheep employing sulphydryl compounds:	
(*a*) Concentrations of aqueous solutions which failed to induce oviposition ...	171
(*b*) Concentrations which induced oviposition	171
3. The incorporation of attractants with sheep wool:	
(*a*) Oviposition on sheep wool	171
(*b*) Trapping experiments using sheep wool and attractants	172
4. Discussion	173
5. Summary	174
References	175

INTRODUCTION

Hobson (1935) showed that a number of agents, the majority possessing the common feature of a high ammonium content will, when placed in the fleece of the living sheep, provoke oviposition by the sheep blow-fly *Lucilia sericata*. Furthermore, he found (1936) that substances such as indole and skatole normally associated with putrefactive processes were also attractants. Experiments were made using these various agents on sheep-skins, but no attraction of the fly resulted, and it was concluded that the living sheep contributed some essential agent (the *S* factor) which, when combined with one of these putrefactive substances (the *P* factor), led to the attraction of the fly and subsequent oviposition.

In the course of the present blow-fly investigations it was found possible to obtain oviposition by *L. sericata* on a clipped fleece by keeping it moist and incorporating an attractant. This result clearly indicated that differentiation of the attractant into a *P* factor and an *S* factor involving a living sheep was not wholly necessary. Evidently, the *S* factor could arise, at least in part, from the fleece alone. It seemed likely that part or all might result from a breakdown of fleece keratin, with the possible production of sulphur-containing compounds from the cystine.

The problem of analysing the agents responsible for attracting blow-flies to the sheep was therefore re-examined and three aspects have been developed as follows:

1. Various materials found by Hobson to be attractants have been chemically analysed for ammonia, indole, skatole and sulphur-containing substances.

2. Field tests have been made on sheep to compare the attractive powers of hydrogen sulphide and ethyl mercaptan with known attractants.

3. Attractants have been combined with sheep wool to develop some method of trapping blow-flies.

1. CHEMICAL EXAMINATION OF ATTRACTANTS

The examination of various materials found by Hobson to be attractants was undertaken to decide how far their enhanced activity after incubation and the attractive power of larval excreta could be attributed to the ammonia content in conjunction with hydrogen sulphide; later some determinations of indole and skatole were added.

Ammonia was determined colorimetrically with Nessler's solution, employing steam distillates from all mixtures (dung, broth, etc.). Sulphydryl compounds were detected by making use of the catalytic decomposition of iodine-azide solution (Feigl, 1937), the evolution of nitrogen being a sensitive test for hydrogen sulphide, although not for ethyl mercaptan. In practice the concentrations were so low that it was not possible to use sodium nitroprusside, but Freudenberg & Münch (1940) have reported an alternative procedure capable of estimating hydrogen sulphide concentrations of the order of 3–8 μmg. With dilute solutions and an excess of iodine-azide reagent the catalytic decomposition ceased, and it was possible to use the resulting colour of the solution for a colorimetric quantitative determination. Solutions were matched against distilled water containing the reagent and compared with standards

of sodium sulphide solution, the hydrogen sulphide content of which varied from 0–2 μmg. per ml. The method gave consistent results, but depended on the absence of substances capable of reacting with iodine, e.g. for a direct estimation with urine the rate of nitrogen evolution was compared with standards.

Very dilute solutions, less than 0·5 μmg. per ml., still showed an obvious gas evolution, and solutions more than 2 μmg. per ml., which completely discharged the iodine colour, were suitably diluted. The calibration was made against sodium sulphide, since aqueous solutions of hydrogen sulphide are most unstable. For this reason, in the field trials the concentrations of hydrogen sulphide were determined on the morning of the actual trial on solutions which had stood overnight, and the values were checked the following day.

The decomposition of the iodine-azide reagent required a much higher concentration of ethyl mercaptan, of the order of a 0·01 % solution (100 μmg. per ml.), which possessed a most distinctive smell, so that it is considered that the iodine-azide effect for distillates was almost exclusively due to hydrogen sulphide. Since a second steam distillation of dilute solutions of both mercaptan and hydrogen sulphide often removed the activity completely, it follows that the sulphydryl content determined must necessarily be well below the actual value.

The procedure for estimating indole and skatole followed that of Rudolfs & Chamberlain (1932), the chloroform solutions from the *p*-dimethylaminobenzaldehyde being compared with standards colorimetrically. Since indole and skatole give red and blue solutions respectively, it was possible by matching the shade to determine approximately the relative amounts in the dung.

(*a*) *Experimental procedure in the chemical examinations*

Ammonia. All mixtures were steam distilled, and the amount of distillate collected varied from 400 ml. to a maximum of 1000 ml., and was dependent on reducing the ammonia concentration below 0·5 mg. per 100 ml. The determinations for ammonia and sulphydryl compounds were done immediately following the completion of the distillation.

Sulphydryl compounds. Portions of 1 ml. of reagent (5·0 g. sodium iodide, 0·500 g. iodine and 5·0 g. sodium azide in 60 ml. water) were accurately pipetted into 10 ml. of distilled water and into 10 ml. samples from a standardized solution of sodium sulphide (sodium arsenite method employed), prepared to contain the equivalent of 0·5, 1·0, 1·5 and 2·0 μmg. of hydrogen sulphide per ml. The solution colours were compared with the water standard in a Leitz colorimeter, capable of readings within a depth of 100 mm., after careful stirring and standing for 10 min. With the water at 30, the above solutions gave readings of 34, 40, 50 and 72 respectively.

The standardized sodium sulphide solution deteriorated on keeping, and an initial concentration giving a reading of 72 had fallen to 57 after 2 days and to 43 after 7 days.

A 0·01 % solution (100 μmg. per ml.) of ethyl mercaptan gave only slight effervescence with a reading of 34, but after standing 3 weeks a reading of 31 was obtained.

Indole and skatole. Two separate 250 ml. fractions of steam distillate were taken, and the next day 200 ml. of each were combined and extracted with ether. After washing with sodium hydroxide and hydrochloric acid the ether was very slowly distilled from 10 ml. of water, then from a further 7 ml. and finally from 5 ml., the combined aqueous solutions being made up to 25 ml. Amounts of 3, 5 or 10 ml. as necessary were compared with standard indole or indole-skatole concentrations employing 1–3 ml. of freshly prepared 0·001 % solutions, each solution being diluted to a standard volume of 10 ml. before adding the reagents and all heated together in the water-bath, then cooled. The solutions were each extracted with 10 ml. portions of chloroform, which were separated, dried over sodium sulphate and the comparison made in the colorimeter.

(*b*) *Results of analysis*

Hobson (1935) tested a series of substances, before and after incubation for 4 days at 37° C., and determined their power of attraction for *L. sericata* by placing 5 ml. (approx.) of an aqueous extract on cotton-wool which was fastened in the fleece of a sheep. He denoted the varying power of attraction by classifying the oviposition as '+', '+ +' or '+ + +' with '−' indicating no eggs laid. The results of his field trials are recorded, along with the analyses made in the present investigation, in Table 1. The table shows that the materials which are attractive are characterized by a substantial increase in the ammonia, sulphydryl and, when present, indole-skatole content compared with the non-attractive form. Considerable variation occurs and the results with scouring dung indicate that its more fluid condition makes it more effective than normal dung as an attractant in the field experiments.

It was not possible to detect indole-skatole in larval excreta, and it is quite evident that the reported activity of the excreta owes nothing to the presence of such putrefaction products. The examination was carried through with an aqueous solution of larval excreta amounting to 400 ml. having a total ammonia content of 0·05 g. Other components of the excreta of *L. sericata* produced in the nitrogen metabolism have been investigated by Brown (1938*a*, *b*).

The field results obtained by Hobson (1936), following the attempted chemical separation of larval excreta, can be readily interpreted if the active agent is a disulphide or sulphydryl compound in conjunction with ammonium carbonate.

Table 1

Substance	Attraction value (Hobson, 1935)		Ammonia mg.	Sulphydryl value mg. H_2S	Indole mg.	Skatole mg.
Larval excreta, 25 ml.	+ + +		Distillation 5·0	None	None	None
			Direct 6·9	0·2	—	—
Each tube contained 5·00 g. minced beef muscle and 5 ml. water, was stoppered with cotton-wool and had been sterilized. Incubation after infection was at 37°C. for 4 days.						
Beef extract broth, sterile	−		1·29	None	None	—
			1·40	None	—	—
Ditto, infected with dung	+ +		35·2	0·20	—	—
			57·7	0·36	—	—
			36·4	0·15	—	—
Ditto, after air infection (slight)	+ +		10·6	0·27	—	—
(heavy)			50·2	0·31	1·36	—
			37·2	0·43	0·86	—
Ditto, infected by larval organism	+ +		67·4	0·34	—	—
			64·0	0·32	—	—
			54·1	0·28	0·83	—
Ditto, infected with excreta	+ +		42·6	0·09	—	—
			36·8	0·09	—	—
			39·4	0·06	—	—
			20·8	0·19	0·02	—
Fresh sheep urine, 25 ml.	−		3·0*	Trace	—	—
Incubated urine, 25 ml.	+		24·0	0·43	—	—
			56·7	0·30	—	—
Dung from scouring sheep, 20 g.	+ +		15·3	0·42	—	—
			20·5	0·55	—	—
			18·5	0·69	—	—
Sheep dung from rectum, evaluated on the day after killing, 20 g.	−	i	16·5	0·43	—	—
		ii	10·7	0·66	—	—
		iii	7·4	0·79	—	—
		iv	7·0	1·98	0·032	0·114
		v	5·3	1·07	0·015	0·053
Incubated dung, 20 g.	+	i	39·3	1·22	—	—
		ii	22·0	1·16	—	—
		iii	29·3	1·27	—	—
		iv	21·1	1·93	0·028	0·276
		v	23·8	1·52	0·111	0·055

The figures (i–v) for sheep dung and incubated dung indicate related samples.
Determinations of indole and skatole were only made in the cases shown.

* In direct determinations of ammonia samples blacken immediately.

2. FIELD TRIALS ON SHEEP EMPLOYING SULPHYDRYL COMPOUNDS

In the main, the method followed was that originated and described by Hobson (1935). Briefly, it consisted of incorporating a known amount of the test substance with cotton-wool and fastening the pad in the fleece of the sheep, which was then given free range, rounded up after an interval of about 2 hr. and any egg batches in the area of the pad counted. In view of the fact that the aim of the present investigation was to determine the minimal effective concentration of a substance, the following precautions were necessary:

(*a*) All liquids used were pipetted as 5 ml. portions of solutions of known strength and were absorbed on pieces of cotton-wool of equivalent size. (*b*) The sheep were numbered and records kept of the results obtained for each sheep. This was very necessary because the threshold value of attractiveness varied with individual sheep and it was essential that the tests were systematically distributed over the flock. (*c*) When more than one trial was made on the same day, no sheep was used in the second and subsequent trials if oviposition had

been induced on it in the first trial. This precaution was taken since on several occasions such a sheep was again struck although the pad carrying the attractant had been removed immediately following the actual trial. (*d*) As proof of fly activity at the time of an experiment, two controls were run employing the minimal concentration of attractant known to induce oviposition.

All the field tests described in this section were made in late August and early September 1943 on 10 wether lambs in an enclosure of about 1 acre on the College Farm at Aber.

(*a*) *Concentrations of aqueous solutions which failed to induce oviposition*

Ammonium carbonate from 0·1 to 0·5 %.

Ammonium carbonate (0·1 %) with from 0·0001 to 0·01 % indole.

Hydrogen sulphide from 0·015 to 0·3 % (0·3 % represents maximum strength of solution obtained by bubbling hydrogen sulphide through water).

Ammonium carbonate (0·1 %) with 0·0002 % hydrogen sulphide.

Ethyl mercaptan from 0·0001 to 0·01 %.

Ammonium carbonate (0·1 %) with 0·0001 % ethyl mercaptan.

Ammonium carbonate (0·1 %) with 0·0001 % ethyl mercaptan and from 0·0001 to 0·0005 % hydrogen sulphide.

(*b*) *Concentrations which induced oviposition*

Ammonium carbonate (0·5 %) with 0·1 % indole.

Ammonium carbonate (0·5 %) with 0·01 % ethyl mercaptan.

Ammonium carbonate (0·1 %) with 0·002 or 0·01 % ethyl mercaptan.

Ammonium carbonate (0·1 %) with 0·001 % ethyl mercaptan and 0·0001 or 0·0005 % hydrogen sulphide.

It is evident that a solution of 0·1 % ammonium carbonate and 0·002 % mercaptan will induce oviposition by *L. sericata* on living sheep, whereas neither component alone is active at these concentrations; the sensitivity of the solution is increased following the addition of traces of hydrogen sulphide.

It is important to note that over the range 0·0001–0·01 % (1–100 μmg. per ml.) ethyl mercaptan alone is unable to induce oviposition and also that, under the same conditions, the active concentration for mercaptan is less than that required with indole.

These results were obtained in spite of adverse weather conditions (cloudy days, maximum air temperature 18–20° C.) at the time the tests were carried out. It is probable, therefore, that under more favourable conditions, lower concentrations will prove capable of inducing oviposition.

3. THE INCORPORATION OF ATTRACTANTS WITH SHEEP WOOL

(*a*) *Oviposition on sheep wool*

A large number of trials were made using approximately 2 oz. portions of sheep wool soaked with substances shown by Hobson to induce oviposition when placed on the living sheep. These pieces of wool were exposed in an area where *L. sericata* was known to occur, but no oviposition was recorded in such experiments. Persistent trials were made using 10–50 ml. of Hobson's standard attractant (1937) consisting of 2 % ammonium carbonate and 0·04 % indole in a vessel surrounded by wool. No oviposition occurred, although the experiments were made under ideal weather conditions in a calorimeter maintaining the wool at temperatures from 20 to 55° C. It must be recorded, however, that on a few occasions there was a definite attraction of *L. sericata* and as many as five females of this species have been observed on the wool at one time.

Table 2. *Oviposition on wet fleeces*

Date (July)	Air temp. °C.	Fleece temp. °C.	No. of wet fleeces	No. of fleeces on which eggs were laid
17	27	—	1	1
18	15·5	18	2	0
24	26	32–39	3	2
*25	21–25	28–37	4	1*
27	19·5–23	25–33	1	1
28	18–25	32	1	1

In the above experiments the attractant was 10 ml. of a solution containing 2 % ammonium carbonate and 0·04 % indole, except in the case * where the solution was 2 % ammonium carbonate with 0·02 % indole.

The possibility existed that the amount of wool might be too small to produce the necessary stimulus for oviposition, so further experiments were made employing whole sheep fleeces. It was found that oviposition could only be obtained when such fleeces were exposed in a moist condition, thus emphasizing the importance of the humidity factor which Davies & Hobson (1935) showed to be essential for the development of eggs and young larvae (see Table 2). Each fleece was soaked in water, spread out on the grass, and in the centre of the fleece was placed a small glass jar containing 10 ml. of the attractant absorbed on a small piece of sheep wool. There was a distance of 6 ft. between fleeces and in all experiments two control fleeces were maintained; one, a wet fleece without attractant, and the other, a dry fleece with attractant. The fleeces varied in weight from 1½ to 2 lb. and, with one exception, were from unwashed sheep sheared on 17 July; the remaining

fleece was from a washed sheep and had been stored for 3 weeks before it was used in the experiments. Such washing and storing does not permanently remove the wool factor, because this washed fleece was the only one active when the attractant contained half the normal amount of indole (see Table 2; 25 July). Oviposition usually occurred on the wool in the jars containing the attractant, but on some occasions batches of eggs were recorded in the fleece adjoining the jar; all egg batches were bred out and proved to be those of *L. sericata*.

(*b*) *Trapping experiments using sheep wool and attractants*

The trap used in this section of the work was designed in the first place for recording the seasonal variations in the blow-fly population, and a detailed description will be given in a later publication (Cragg & Hobart). Briefly, it consists of coarse-mesh mosquito netting in the form of a cylinder about 1 ft. 9 in. high and 11 in. diameter, and within the cylinder is a cone of the same material, the apex forming the fly entrance. This trap is supported from a wire framework. In the experiments to be described, the trap was placed over a small glass vessel (4 × 2 in. diameter) containing the attractant solution, the vessel being surrounded by ½ lb. sheep wool.

The number of tests made at any one time varied from 1 to 6. The trapping positions were fixed, no two traps being closer together than 10 ft., and the actual positions used on any one day depended on fly activity in the neighbourhood. Considerable variations were found between various trap positions and different portions of fleece, and when it was clear that so many factors were involved these were standardized as far as possible and attention was concentrated wholly on the examination of solutions of ammonium carbonate, indole, hydrogen sulphide and ethyl mercaptan, either alone or in various combinations. All attractant solutions were freshly mixed before each test. Portions of fleece used in the experiments were tested without any attractant present, in order to make certain that the fleece alone possessed no attractive power.

The duration of each test varied, but the majority represented 2–3 hr. exposure, although on days when fly activity was intense the traps would be changed sometimes after an interval of an hour.

The results obtained during the period 17 August–21 September are summarized in Table 3. During this period the blowfly chiefly attracted to the baits used was the female of *L. caesar*. Usually the other flies (mainly small Anthomyids) formed 0–6 % of the catch, so it is evident that the type of trapping medium used proved to be a specific attractant at that time of the year for *L. caesar*. For the purpose of this investigation the term *L. caesar* also includes *L. illustris* and *L. ampullacea*, since the females of these three species cannot be distinguished. However, meat traps operating over the period showed, in the case of males, 1·6 % *L. illustris* and 0·2 % *L. ampullacea* against 98·2 % *L. caesar*; hence it appears reasonable to assume that females of *L. caesar* were also predominant.

At the time of the above trials the population of *L. sericata* active in the neighbourhood was small, since two traps baited with guinea-pig carcasses during the period of the experiments showed a total catch of 51 male and 314 female *L. sericata* against 864 male and 6521 female *L. caesar*. It is possible, therefore, that under more favourable conditions the trapping medium may prove of some value as an attractant for *L. sericata*, and one trial, not included in Table 3, lends some support to this statement. On 27 July (air temp. 19·5–23° C.) the attractant used was 10 ml. 10 % ammonium carbonate, 5 ml. 0·12 % indole and 5 ml. saturated hydrogen sulphide, and out of a total catch of 55 flies, 25 were *L. sericata* with one *L. caesar* and the remainder were mainly Anthomyids. This same mixture used from 17 August failed to attract *L. sericata*, so it is evident that this particular result cannot be due solely to the concentration of the various substances in the attractant.

Freney (1937), in a very comprehensive treatment of trapping experiments, has commented on the difficulty of obtaining comparable results under field conditions where changes in the fly population and weather conditions introduce so many variables; to those discussed by Freney must be added another variable which occurred in the present investigation. This concerns the attraction set up by the flies themselves once inside a trap of the mosquito-netting type. Where large catches were recorded (50 or more flies) they could be used as bait in a trap, replacing both attractant and fleece, e.g. 34 females of *L. caesar* were caught using as an attractant a trap containing 150 live female *L. caesar*. This attraction may result from the flies carrying small amounts of the attractant into the traps.

In view of the many undetermined factors which must have exerted some influence on these field observations, it is obviously unjustifiable in the present state of the work to make any strict comparison between one attractant and another, apart from emphasizing the fact that where mixtures containing hydrogen sulphide are used, attraction is greater in those cases where the hydrogen sulphide is kept in a separate container than where it is mixed with the other constituents. For the present it is concluded therefore that the substances described in Table 3 will, when used in conjunction with a portion of sheep wool towards the end of the

blow-fly season, act as attractants for females of *L. caesar*.

In spite of the number of *L. caesar* trapped by various substances, only in four cases did the flies oviposit in the jar containing the attractant or on the adjoining fleece. It is clear, therefore, that the attractant used is not one which brings about the full oviposition response of *L. caesar*.

During the period 13–21 September, ethyl mercaptan was tested, but neither alone nor in conjunction with sheep wool did it attract flies. Experiments were then made comparing the complete mixture with and without 10 ml. of 0·01 % mercaptan solution, sheep wool being present in both cases. The trials were made at two trapping positions, and on alternate days the two mixtures were interchanged, but with the fleece portions left unchanged. Over a period of 8 days, 615 female *L. caesar* were caught in the trap with ethyl mercaptan present against 361 in the other trap. These differences are not however significant when checked by the '*t*' test, and further trials are obviously necessary. It should be noted that two female *L. sericata* were caught during these trials, both of them in the traps which incorporated mercaptan.

Extensive chemotropic tests in South Africa made under laboratory conditions by Hepburn & Nolte (1943) on *L. cuprina*, showed that ethyl mercaptan alone or in combination with indole was not attractive. This result is the more remarkable, since Freney (1937) in Australia recorded in field trials that ethyl mercaptan alone was definitely attractive to blow-flies, and that it appeared to have the particular power of attracting flies into the vicinity of a trap.

Table 3. *Trapping experiments combining sheep wool with ammonium carbonate, indole and hydrogen sulphide*

Attractant used with ½ lb. sheep wool	No. of trials	Total number of flies caught: *L. caesar* females	Total number of flies caught: *L. sericata* females	Individual catches *L. caesar* females
20 ml. of 0·12 % indole	6	124	0	0, 99, 1, 18, 0, 6
20 ml. of 10 % ammonium carbonate	4	36	0	0, 1, 17, 18
20 ml. saturated hydrogen sulphide solution	7	143	0	80, 41, 18, 1, 0, 0, 3
5 ml. 0·12 % indole, 10 ml. 20 % ammonium carbonate, 5 ml. water	9	206	3	0*, 21, 2, 1, 1, 101, 14, 6, 60
10 ml. 10 % ammonium carbonate, 5 ml. saturated hydrogen sulphide, 5 ml. water	11	442	2	0, 21, 54, 106, 40, 25, 42, 41, 5, 9, 99
10 ml. 10 % ammonium carbonate, 5 ml. 0·12 % indole, 5 ml. saturated hydrogen sulphide	10	169	1	15, 2, 28, 26, 6, 3, 40, 11, 9†, 29
10 ml. 10 % ammonium carbonate, 5 ml. 0·12 % indole, 10 ml. hydrogen sulphide (separate container)	11	424	0	5, 30, 3, 15, 7, 22, 124†, 87, 7, 58, 66
10 ml. 20 % ammonium carbonate, 5 ml. 0·12 % indole, 10 ml. hydrogen sulphide (separate container)	26	1472	19	27, 1, 27, 52, 1, 102, 130, 29, 6, 52, 165, 80, 81, 0, 44, 8, 85, 15, 119, 12, 35, 21, 9, 118‡, 212, 41

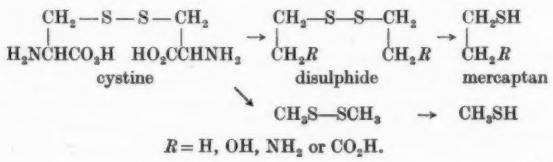
* Oviposition by *L. sericata*. † One male *L. caesar* recorded. ‡ Two male *L. caesar* recorded.

4. DISCUSSION

The work described in sections 1 and 2 clearly suggests that sulphydryl compounds may play an important part in the production of blow-fly strike on sheep under natural conditions. If that is the case, then the most likely source of such compounds would be the cystine in fleece keratin. That the breakdown of keratin could take place finds much support in the literature of the subject. From observations by von Brunswik, referred to by Mark (1925), it appears that 45 % of normal fibres show localized damage. From the summary by Freney (1940) of chemical investigations on the fleece of the sheep, the sulphur content of the wool varies markedly, not only in different regions but even along the staple. Irradiation of wool in dry nitrogen has been shown by Harris & Smith (1938) to produce hydrogen sulphide, but in moist air oxidation ultimately gave a considerable amount of sulphuric acid. Routh (1940) observed a loss of cystine sulphur during prolonged grinding of wool, probably the result of oxidation, since after grinding more than 50 % of the water-soluble sulphur was inorganic sulphate. On the other hand, suint analyses show a relatively low sulphate content (about 3 % of suint

ash, whereas chloride is about 4 %), which suggests that volatile sulphur compounds in very low concentration might be lost through the wool atmosphere. Numerous investigations have dealt with the elimination of hydrogen sulphide from cystine on alkali treatment, indicating the labile nature of the sulphur. There does not, however, appear to be any evidence of mercaptan formation, but the main difficulty may well be the detection of traces of such compounds.

Bacterial action can doubtless play an essential part in the condition of the fleece as is evident from Seddon's observations (1931) on the occurrence of 'water rot' in Merino sheep. He showed that where the wool becomes very wet and has no chance to dry, a bacterial infection may develop which results in the sheep becoming liable to blow-fly strike. It is also significant, from the viewpoint of the present investigation, that he observed that such infection led to the wool fibres being damaged. The present work on sheep fleeces clearly emphasizes that the high humidity which Davies & Hobson (1935) demonstrated as vital for a successful myiasis, also conditions attraction and subsequent oviposition. Studies of bacterial decomposition of cystine under anaerobic conditions have been described, which show elimination of equivalent amounts of ammonia and hydrogen sulphide (Desnuelle & Fromageot, 1939). It is possible that decomposition by bacteria may also produce disulphides or mercaptans, as outlined below, together and carbon dioxide.



R = H, OH, NH_2 or CO_2H.

In the field, under aerobic conditions, the active odour may arise from only one or a combination of these sulphur compounds, and this aspect will be covered by subsequent trials. From personal observations in the field it is certain that the factors produced by the living sheep, whether by bacterial decomposition alone or supplemented by irradiation of the fleece, are far more effective in producing attraction and oviposition than has yet been achieved by a clipped fleece incorporating attractive chemicals.

The trapping experiments have yielded the important result that it is possible to obtain specific attraction, not only of one species *L. caesar*, but of one sex, thus indicating progress in the development of a chemical bait for attracting blow-flies. Since *L. caesar* was caught and not *L. sericata*, it is obvious that at the time there were factors operating which are not yet understood; no attempt is, therefore, made to correlate these results with the rest of the work.

The hypothesis provides no explanation of the role of indole and skatole, but from the distribution of these substances in attractants it appears unlikely that they can play any principal part in naturally induced strike. It is possible, however, that their efficiency as attractants is related to their power as fixatives, which earn them an important place in perfumery. Thus indole may well 'fix' an odour developed in the fleece and now considered to be that of mercaptan or the corresponding disulphide, combined in certain circumstances with ammoniacal products.

5. SUMMARY

1. Oviposition by *L. sericata* has been induced on a moist clipped fleece by incorporating ammonium carbonate and indole, hence attraction is not completely dependent on some factor produced by the living animal.

2. *Chemistry of attractants.* A new method for determining small amounts of hydrogen sulphide is described. Larval excreta contains hydrogen sulphide but no indole or skatole. Following incubation the ammonia and sulphydryl contents of various natural attractants are markedly increased.

3. *Field trials on sheep.* 0·002 % ethyl mercaptan in conjunction with 0·1 % ammonium carbonate was able to induce oviposition by *L. sericata*. The mercaptan concentration could be reduced to 0·001 %, providing 0·0001 % hydrogen sulphide was present. 0·01 % mercaptan and aqueous solutions of hydrogen sulphide alone are not attractive.

4. The results obtained support the hypothesis that attraction is the result of the breakdown of cystine, with the production of sulphur compounds, coupled with the presence of ammonium carbonate (or of some putrefactive material).

5. *Chemical baits.* During the period 17 August–21 September 1943, various combinations of ammonium carbonate, hydrogen sulphide and indole in conjunction with sheep wool proved to be attractants for females of *L. caesar*.

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THE GENUS *KUHNIA* N.G. (TREMATODA: MONOGENEA)

AN EXAMINATION OF THE VALUE OF SOME SPECIFIC CHARACTERS, INCLUDING FACTORS OF RELATIVE GROWTH

By NORA G. SPROSTON, *Keddey Fletcher-Warr Student, University of London*

(With 7 Figures in the Text)

CONTENTS

	PAGE		PAGE
Introduction	176	Systematics	188
Kuhnia scombri (Kuhn)	176	Discussion	188
Kuhnia minor (Goto)	181	Summary	189
Kuhnia macracantha (Meserve)	184	References	189
Heterauxesis in *Kuhnia scombri*	184	Summary of specific diagnoses in the genus *Kuhnia*	190
Allomorphosis in the genus *Kuhnia* ...	185		

INTRODUCTION

The fact that in Monogenea, after a very brief free-swimming larval phase, the body form remains essentially unchanged throughout life, but that it continues to grow very considerably after first attaining sexual maturity, is a commonplace, though its implications are not always fully realized. Many species have been separated mainly on the basis of size. Moreover, the proportions of parts to the whole are, by themselves, misleading because the various parts grow at different rates: so that those species which have been founded on a few individuals differing only in size and proportions of parts from related species must be regarded as provisional. Metrical factors are, however, as important as qualitative characters in diagnosis, and in closely related forms may be the only means of expressing specific differences. When a large series of growth stages is available, the coefficients of differential growth can be obtained for the various parts in relation to the body as a whole or to some convenient part of it: thus providing a quantitative description, if necessary, for intermediate growth forms of a species.

A study of a large series of specimens of the common monogenean from the gills of the mackerel, taken at various times between March and December (1939–42) from the west English Channel area, has revealed the importance of considering relative growth when describing species of Monogenea. In the following account some attempt has been made to assess the value of some characters usually regarded as of specific importance, and of those which actually serve to separate it from its allies: these include a rare species, found for the first time outside Japanese waters. A new genus has been created for these monogeneans from mackerel, and its relationships are discussed.

Kuhnia scombri (Kuhn, 1829) n.comb.

Fig. 1 shows selected examples of the variations in size and form met with in *Kuhnia scombri*. It is at once obvious that the length of the haptor increases more slowly than that of the body, and the body length more slowly than the width, especially in the second half of life. The width is largely dependent on the development of the testes and vitellaria; but the clearly marked alternation of male and female phases found in this species is responsible for the different appearances of the worms figured.

There appears to be no mention of an alternation of sex phases in the literature on Monogenea; though, as might be expected in hermaphrodite animals, some separation of the periods of maturity of ovary and testis would not only reduce the physiological drain on the organism as a whole, but would also minimize the chances of self-fertilization where there are no structural features to obviate this. Some genera show a more marked separation of the sex phases than others—but it has been found in all cases where a sufficiently wide size range has been examined—and it so happens that *Kuhnia* is the most striking example met with so far. Johnston & Tiegs (1922) showed that in young *Lepidotrema therapon* (Gyrodactyloidea), the testis was mature, and proportionally very much larger than the ovary, than in larger specimens: in these the testis was spent and the ovary mature, while the vesicula seminalis was highly distended with sperm and prostatic

glands had become enormously developed (see Sproston, 1945, Fig. 28 *a, f*). This, however, only indicates a single alternation, and there is no evidence that the genital products are shed at different times. A similar state of affairs has been found recently for *Udonella caligorum* (Capsaloidea) (see Sproston, 1945, Fig. 38 *a, c, d*), though in this species it seems probable that the sperm is not stored as in the previous example, as no distended vesicula seminalis is visible. But in *Plectanocotyle gurnardi* and *Microcotyle donavini* (Diclidophoroidea) (see Sproston, 1945, Figs. 95 *a, i–m*, and 101 *c–e*, respectively), like *Kuhnia scombri*, there is probably a repeated alternation of sex phases throughout life giving rise to very different appearances in internal structure, and in shape of body due to the enlargement of different organs during their phases of activity, which during their quiescence are often difficult to detect. This has lead to many misleading descriptions of types.

Fig. 2 is an attempt to show the distribution of sex phases in 6 mm. length groups (see p. 184), in *K. scombri*. It is not possible to determine satisfactorily how many alternations occur, but there are probably at least three male phases and two female phases. In all genera so far studied the testis becomes mature before the ovary.

This species was first described by Kuhn in 1829 as *Octostoma scombri*, and though he figured the haptor as the head and the eight attachment organs as mouths, his observations on the living worms made him sceptical to this current belief that they were attached by the anterior end. In 1894, Goto gave a detailed account of the internal anatomy of the species (as *Octocotyle scombri*), apparently from a few medium-sized specimens: this is in substantial agreement with the present findings, though Goto and other authors have been misled in regard to the structure of the attachment organs, or clamps, which is here given a new interpretation.

The smallest worm found was 1·32 mm. long and the largest 6·50 mm. long: but those less than 1·8 mm. long are considered as late larvae, since most of those greater than this showed definite signs of developing testes. For convenience in comparison with other species the series has been divided

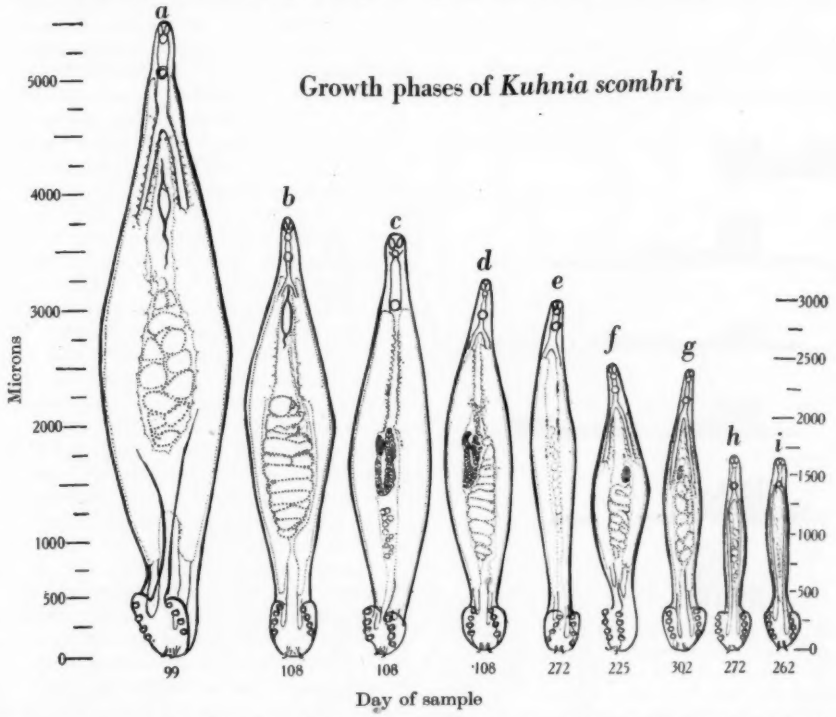

Fig. 1. *K. scombri*. *a–i*, a selection of specimens to show extremes of variation during growth. Note the alternation of sex phases and the differential growth of the parts leading to widely different proportions: all ventral aspect. Specimen *f* shows a rare case of *situs inversus*. (Original.)

into three groups: larvae up to 1·8 mm., those in the first half of growth up to about 3·6 mm., and those in the second half of growth up to about 6·5 mm. (see summary of specific characters, p. 190).

The body is lanceolate, tapering towards a nearly parallel-sided anterior portion which is about a fifth of the total length in young worms to a seventh in larger ones. Posteriorly the body becomes thicker; it is widest about the middle and tapers again to the region just in front of the heart-shaped haptor, which in larger worms is slightly tilted to the plane of the body. As growth advances the lateral edges

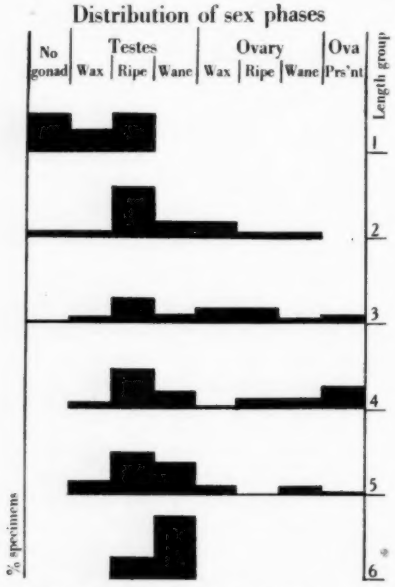

Fig. 2. The distribution of sex phases in the 6 mm. length groups of *K. scombri*. This shows at least three male and two female phases: ripe gonads at a maximum in group '2', and '4' to '5'—smaller sub-divisions would show this more clearly, for most of the larger fraction of group '5' had less active gonads than the smaller fraction.

of the posterior third of the body tend to grow ventrally and slightly inwards, so that in the larger specimens these folds are continuous with the anterior edge of the haptor (Fig. 1*a*).

The clamps are arranged in two slightly divergent rows on either side of the haptor—the anterior pair being nearest together and the last pair almost on the margins: the last pair is placed about a third of the haptor length from the end in larger worms, and about a quarter length from the end in the smaller group. The four pairs of clamps are nearly all of the same size, though there is some individual variation, the first and last pairs often being slightly smaller than the other two. In larval worms the anterior pair can often be seen in formation, still below the surface of the anterior margin of the haptor, only very slightly cuticularized and much smaller than the others. In Fig. 3 (*j*, *k*, *l*) is shown a clamp which has been dissected out from the haptor: it is formed of a very thin cuticular sac open posteriorly, a more heavily cuticularized band is laid down near the edges of the sac in the form of two U-shaped pieces which are joined anteriorly, the two loops being really a continuous flattened band and the twists at the two shoulders form springs tending to open the sac, or rather, to cause the ventral loop to spring outwards, since the dorsal loop is attached to the ventral face of the haptor. The separate loop within the sac (= middle piece) is shown in two positions nearly at right angles in Fig. 3 *k*, *l*; it is scoop-shaped and heavily cuticularized only along the edges, it is freely articulated by the thickened upper ends, under the shoulder-springs of the double loop (*j*). The thin triangular centre portion has a triangular hole through which is threaded the T-shaped expanded tendon of the main retractor muscle bundle of the clamp (Fig. 3 *i*). this latter enters the capsule through a polygonal hole in the dorsal wall near the edge where it is attached to the middle of the dorsal loop (*j*); in dorsal view its attachment along the capsule wall is seen as parallel rows of small thickenings (*h*, *q*, *r*, *s*). The action of the clamp is that when the muscle contracts, the tendon sliding in the slot of the middle piece draws the latter towards the dorsal loop, and the ventral loop moves with it, because the middle piece is clipped to it along the posterior edge by the incurved flange-like margin (see Fig. 3): thus the three pieces are drawn together and the capsule is closed on a portion of gill tissue of the host. When the muscle is relaxed, the shoulder springs are free to release the ventral loop and middle piece, which moving away from the dorsal loop, open the mouth of the capsule and so release the hold on the gill tissue. The structure is not always easy to make out *in situ*, and in life the cuticularization is not well differentiated from the surrounding prismatic fibres; various aspects of the clamp are shown in Fig. 3. The 'cross-bar' on the anterior edge of the clamp, mentioned by various authors, is only the optical section of the thin cuticular capsule. The sclerites forming the clamps are somewhat elastic, so that different states of contraction and fixation produce some slight variation in shape: this is usually almost square, though the longitudinal axis may be elongated. For purposes of comparison the size of the clamps has been expressed as the square root of the product of the two axes, at least four clamps being measured for each worm, and the mean of these taken: in larvae they measure 35 μ, and in adults increase from 42 to 52 μ.

Two anchors project from the hind margin of the haptor (Fig. 3*f*), the shafts are solid and ridged—the imprints of the prismatic fibres which produce the spur is at about mid-length in larvae and small worms, and in older ones about three-fifths from the proximal end. The anchors in larvae are 100 μ

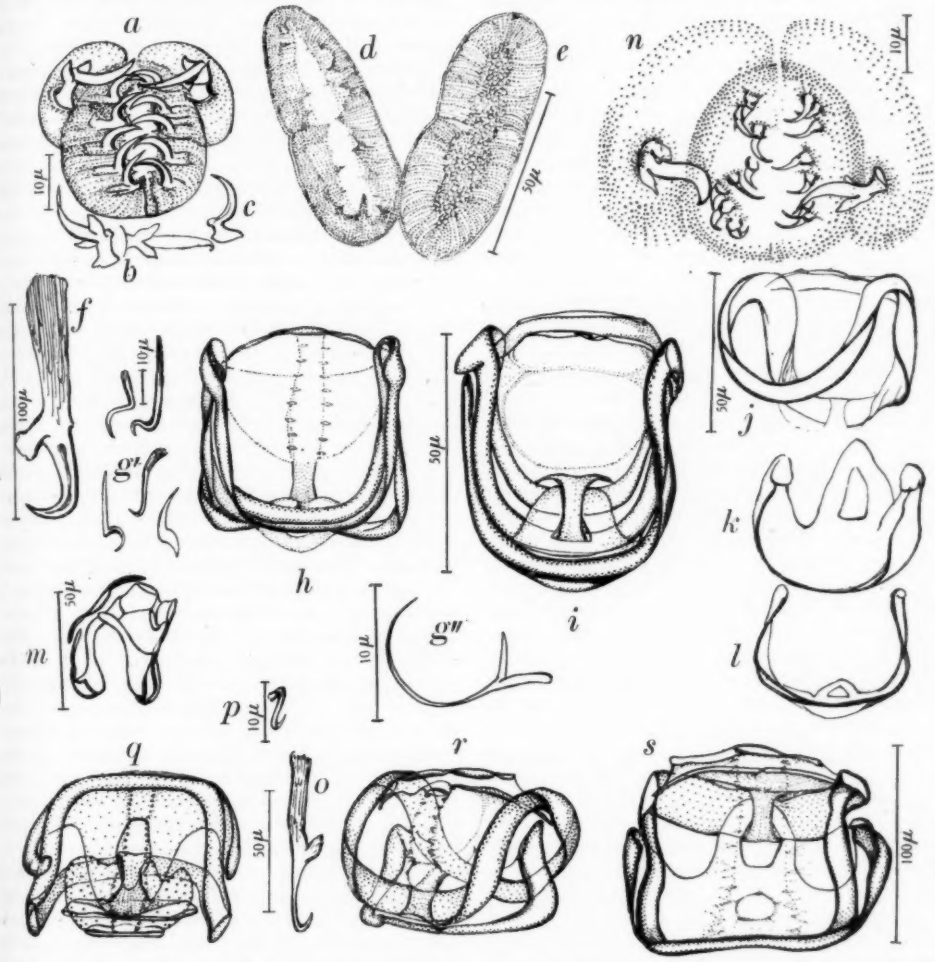

Fig. 3. *a–m, K. scombri*; *n–s, K. minor* (cuticularized structures). *a*, genital pore and hooks *in situ*, ventral view; *b*, hooks of antero-lateral pair—two views; *c*, hook from the inner longitudinal rows; *d*, *e*, surface and deeper focal views of oral 'sucker' showing the arrangement of the refractile prismatic fibres; *f*, large anchor; *g′*, various aspects of small anchors; *g″*, larval hooklet from haptor; *h*, clamp, closed, dorsal aspect; *i*, clamp partly closed, ventral aspect; *j*, ventral and dorsal loops disarticulated, ventral view; *k*, *l*, middle piece from two ventral aspects—disarticulated; *m*, clamp, partly open, profile; *n*, dorsal view of genital pore and hooks *in situ*; *o*, large anchor; *p*, small anchor; *q*, clamp which has been swung forwards, partly open, showing loops and inverted middle-piece from dorsal aspect; *r*, clamp closed, dorsal view; *s*, clamp open, ventral view. (Original.)

them—but the free, heavily sclerosed hook is hollow; the stout spur to which the retractor muscle is attached is situated on the side towards the distal curvature. The shaft grows during adult life so that long, in small adults 102–105 μ, and in older ones 112–120 μ.

Between the anchors there is a pair of small twisted hooks with sickle ends, rather long shafts

and curved bases; these are very difficult to measure owing to the ends being in a different plane to the shaft—their apparent length is about 20 μ (Fig. 3*g'* represents camera-lucida drawings of five of these from different angles). These small hooks are easily lost, and Goto (1894, p. 43) did not see them. In some specimens additional, smaller, hooks are found (Fig. 3*g''*), but they are so delicate that they can only be seen in abnormally flattened specimens. A pair is sometimes found between the anchors and small hooks, and another is, more rarely, found midway between the anchors and the last pair of clamps: they are almost semicircular with bifid base, and the length, not counting curvature, is about 12 μ. It is thought that they are the persistent larval hooklets, similar to those described by Remley (1942) on the larval haptor of *Microcotyle spinicirrus*, but this is the first time they have been found in a member of the family Mazocraeidae.

The oral suckers are elongated oval depressions in the posterior dorsal wall of the buccal cavity. They are also formed of refractile prismatic fibres so that they do not take up stains in the fresh condition; of themselves they are not contractile, but they change shape and orientation slightly owing to several muscle bundles which are attached to them (Goto, 1894, pp. 17–18, 22, Pl. IX, fig. 8). In Fig. 3, *d* represents one in surface view showing the radially disposed fibres withdrawn rather irregularly round the lumen (in some specimens this gives the appearance of a bilocular sucker similar to that described for some species of *Microcotyle*); *e* shows the neighbouring sucker of the same worm in deeper focus, the roof being composed of the closely packed ends of the prismatic fibres.

The mouth leads directly into an almost spherical pharynx from 30 to 50 μ in diameter. The oesophagus is broad and extends through the narrow anterior region of the worm, at the end of which it bifurcates into the two intestinal crura. These are clearly visible as unbranched tubes in the larvae, but in older worms they become increasingly branched and overlain by the vitellaria; the rami are short and wide between the successive dichotomies. These occupy a wider area on the outer side of the crura than on the inner, and though the inner rami approach each other behind the testes in larger worms, no anastomoses have been detected. The branching ceases in front of the haptor at a distance about equal to its length, and the two wide crura extend into the haptor to varying levels; quite frequently one crus is longer than the other, the left usually being the longer, though this is not constant (Fig. 1). In the larger specimens there are usually numerous black pigment granules laid down in the walls of the intestinal rami, and these are thought to be derivatives of the haemoglobin of the host since they increase with age; a theory supported by the fact that they are particularly noticeable in some younger worms which when collected were clearly feeding directly on host blood, for they were scarlet in colour. Usually the younger worms only feed on the gill epithelium and mucus, and they are not tinged with red, and these have few granules, and not infrequently the granules are absent. Chemical tests on formalin-preserved worms, using the technique of Brown (1911 *a*, *b*) were negative; so that a demonstration of the nature of the pigment awaits fresh material.

The gonads occupy nearly the whole of the intercrural area in the middle third of the body, but their relative proportions vary very widely according to the phase of development. In the youngest worms showing developing gonads the testes are always the first to form: they occur as about a dozen rounded bodies, usually the five anteriormost are smaller and lie alongside the rudiment of the oviduct, to the left of the mid-line. They increase in size and by their mutual pressure become transversally elongated masses, occupying the third and fourth fifths of the body. Meanwhile the worm has increased in width rather more rapidly than in length. As the testes become spent the regular transverse boundaries seem to break down and the whole testicular mass appears as a continuous zigzag skein, the parts now being irregular polygons and highly vacuolated. During the waning of the testis the ovary begins to develop, first as a small darkly staining punctate body just above the first testis, on the right of the mid-line: it is 160–180 μ long and 100 μ wide. The developing ova grow and are pushed out into the proximal part of the oviduct, which is a wide tube bent on itself in the mid-line of the body where it occupies the fourth sixth of the length in medium-sized worms. The maturing ova do not develop further until the testes have become spent: this is the end of the first male phase, seen in worms between about 2·5 and 3·5 mm. long.

When the testes are waning at the beginning of the female phase the Y-shaped vitelline duct becomes conspicuous, as it fills with minute refractile granules; it lies in the middle line in front of the oviduct, and is about the same length as the latter. Parallel to the vitelline duct, on its right side, is the long vitello-intestinal canal which opens into the right crus of the intestine a little behind the level of the origin of the right branch of the vitelline duct. The female phase begins by the distal, ascending, portion of the oviduct receiving developing ova which are being pushed up from the descending portion. These ripe ova must be shed very quickly as it is extremely rare to find a worm with a mature egg in the uterus as well as ova in the oviduct, and yet on only one occasion (out of some 250 specimens) has more than one mature egg been seen in the uterus at one time. Usually when mature eggs are

found in the uterus there is no trace of either oviduct or ovary, spent testes, or testes beginning to reconsolidate, being the only gonads present. Worms at the end of the first female phase (containing eggs) are about 4 mm. long.

In the subsequent male phases the testes occupy a wider area than in the first one, and the areas covered by vitellaria are also wider, the acini being more crowded between the intestinal rami as well as above and below them (see Fig. 1*a*); dorsally the vitellaria extend as far forward as the genital pore, half-way along the oesophagus, but ventrally they may not reach as far as the intestinal bifurcation. They lie closely round the gonads, and in larger worms are confluent for a short distance posterior to the testes, thence they continue to overly the crura until the ramifications cease. The largest size-groups (4·8–5·8 and 5·8–6·8 mm.), which were found in the early part of the year, were nearing the end of a female phase and a male phase, respectively, and the latter invariably showed degenerative changes in the vitellaria as well as having large irregular vacuoles in the testicular masses: the vitelline duct, and often the vitello-intestinal canal, being distended by large globules of a dark yellow highly refractile substance.

The common genital pore is in the mid-ventral line, usually about half-way between the pharynx and the intestinal bifurcation. It receives the uterus, which must also function as a vagina, Goto (1894, pp. 123, 203) having shown that this is absent as a separate canal. At the base of the genital atrium is the genital bulb formed of prismatic fibres with underlying powerful muscles; it varies from 25 to 30μ in diameter according to the size of the worm, with two longitudinal rows of five sickle-like hooks which are arranged in an alternating manner on either side of the longitudinally opening male duct (Fig. 3*a*). The genital bulb has two accessory reniform lobes situated antero-laterally, each bearing a strong hook of a distinctive type. It has a stout base bearing two broadly pointed projections on either side of the blade, which is broad and curved outwards, ending in an abrupt point; this is 13·3μ long and the base 8·3μ (mean total 21·5μ in length). The small hooks have the following average measurements (not accounting for curvature): blade 9·5μ, base 7·6μ, total 17μ in length. Their number is slightly variable: Goto (1894, p. 204, and 1898, p. 275) found only four pairs, though Dujardin (1845, p. 313, Pl. 8E) gives a good figure of the genital bulb showing five pairs. In the present samples, fifty worms taken at random had the following numbers of inner hooks: 5+5, 74%; 6+4, 10%; 4+5, 6%; 4+4, 40%; so that 16% showed fewer than the normal number, but the assumption is that in these worms the loss is traumatic, probably suffered during copulation, or less likely, at the hands of the collector, because these hooks do not project above the surface of the body.

The eggs are relatively large, fusiform, operculated and bear a simple filament at each pole. Contrary to what is generally assumed, the egg size in Monogenea is not constant within the species, and should not be used as a primary diagnostic criterion. Gallien (1935, p. 53) gave a widely varying series of measurements for the eggs of *Polystoma integerrima*, and Remley (1942, p. 148) found that the rather smaller degree of variation in *Microcotyle spinicirrus* was due to the size of the worms, larger worms producing larger eggs. In *Kuhnia scombri* a series of eighteen measurements* shows a degree of variation in egg shape and size at least equal to that found by Gallien: a selection of outlines of half-eggs is shown in Fig. 4. No correlation between egg size and size of worm, or season of production (i.e. first or [?] second female phase) could be found. Of the complete eggs in Fig. 4, the two upper ones are spring eggs ([?] second female phase) and the two lower are autumn eggs (first female phase). The suture of the operculum occurs between a third and a fifth of the length from the anterior pole. When the egg is laid the segmentation has apparently not begun, as only one large nucleus could be seen. Development occurs after it has become attached to the gill filaments of the host, by means of the coiling polar strands. By analogy with *Microcotyle* (Remley, 1942, pp. 149–50) the ciliated embryo which hatches out is the stage at which active migration from host to host takes place; though the young post-larvae of *Kuhnia scombri* are very active, and it is possible that they too make their way from fish to fish when these are swimming in close formation.

Kuhnia minor (Goto, 1894) n.comb.

Goto (1894, pp. 203–6) described two species of *Kuhnia* from the gills of *Scomber japonicus* from Japan: a larger one ('about 4 mm. long') which he

* Egg measurements (in μ) of *Kuhnia scombri*:

																				Means
Length	312	300	295	285	275	275	270	262	247	235	232	230	230	230	210	205	200	194		*250*
Width	82	80	103	126	110	91·5	110	87	110	72	83·5	75	63	53·5	49	71	90	106·5		*87*
Filaments:																				
Anterior	305	300	475	390	300	165	124	140	230	180	285	172	—	—	—	170	210	194		*243*
Posterior	180	165	470	168	213	180	76	126	108	95	230	100	305	—	—	190	303	106·5		*188·5*

Measurements were taken from the inner side of the shell. The shell thickness varies from 3 to 6μ and becomes hollow in the basal parts of the filaments.

named *Octocotyle major*, and a smaller one ('about 2 mm. long') as *O. minor*. When examining *Scomber scombrus* at Rhode Island (North American Atlantic), he found further specimens of his larger species which he considered later (1898, pp. 274–6) to be conspecific with *Octocotyle scombri* (Kuhn). In both his papers he questions the identity of specimens described by European authors as *O. scombri* (Kuhn), and is inclined to divide them between his two species on the basis of size, presence of one or two pairs of terminal hooks, and the number of hooks on the genital bulb. He defined *O. scombri* [= *O. major* Goto] as having only one pair of terminal hooks (with hollow ends) and a total of five pairs of genital hooks, and *O. minor* with two pairs of terminal hooks, a larger (wholly solid) outer pair and an inner, much smaller, filiform pair, and the genital hooks numbering six pairs.

It may be that Goto was misled as to the significance of the specific characters he used owing to the paucity of material (for he admits that his drawing of *O. major* (1894, Pl. 9, 1) was of a specimen distorted by over-compression), and that in his worms the second pair of terminal hooks and two of the genital hooks were lost. However, it is certain that *O. major* Goto is a synonym of *Kuhnia scombri* (Kuhn), and that *Octocotyle minor* Goto is a valid species described by him for the first time, not, as he thought, confounded by previous authors with *O. scombri*.*

Among the specimens in the present collection from the gills of *Scomber scombrus* are three which have been assigned to *Kuhnia minor* (Goto), for in spite of the great discrepancy in size (two of them being larger than any recorded specimen of *K. scombri*) they show all the outstanding characters of Goto's species. Incidentally, this illustrates the malpractice of emphasizing mere size as a specific character in monogenetic trematodes; Remley (1942) gives a striking instance of wide size range in *Microcotyle spinicirrus*, which grows to 13 mm. long, yet he found worms of only 2 mm. already producing mature eggs.

Specimen 'A' (Fig. 4—this has been slightly distorted in mounting) and a similar damaged one of about the same size (7 mm.) were found in March (day 99), and specimen 'B' (3·85 mm.) at the end of October (day 302): both in company with *Kuhnia scombri*. Fig. 4 'C' is copied from Goto's drawing of *Octocotyle minor* (1894, Pl. 9, fig. 7), which according to his scale is 1·7 mm. long, and found by him at Hagi and Misaki, Japan; so this is only the second record for the species, and the first from *Scomber scombrus*. It appears to occur about a hundred times less frequently than *Kuhnia scombri* on the gills of mackerel in British waters.

The most obvious specific difference from the genotype is that the body does not become tapered posteriorly to the region just in front of the haptor, so that the latter is continuous with the body and the four pairs of clamps have intestinal rami and vitellaria on their outer sides and the last pair is on the hind edge of the body. The clamps themselves are on the same plan as those of *K. scombri*, but they are always broader than long, and in medium and large worms they are much larger and the sclerites altogether stouter than those in *K. scombri* (Fig. 3 *q*, *r*, *s*). The anchors, or large hooks, are differently shaped, more slender, and are, as Goto stated, wholly solid (Fig. 3 *o*); they measure 75 and 53·2 μ respectively in 'A' and 'B' (37 μ in 'C'), their small absolute and relative size being compensated for by the greater development of the clamps. The small hooks were lost in 'A', but in 'B' they were present, being twisted as in *K. scombri*, but apparently lacking the sickle-shaped ends: they appear to be about 9·5 μ long (Fig. 3*p*). Goto figures them (Pl. 9, fig. 9*b*) as very thin and only slightly curved spines.

* Layman (1930, pp. 22–3, 54–5) gives some data on the parasites of *Scomber japonicus*, among which he describes *Kuhnia scombri*. Since this is not easily available, and is in Russian, a translation is given here, for which I am indebted to Mr I. Zacopanay, B.A., of Cambridge:

'Length 5·408–1·802 mm., width 0·655–0·344 mm. Oral suckers 0·327–0·360 mm. long and 0·229–0·245 mm. wide. The disposition of the hooks round the genital pore is different from that described by Dujardin, in that the inner hooks are joined posteriorly, so as to form a semi-circle. The posterior attachment apparatus [= clamps] are not completely sessile as described by other authors, but are on separate humps. The hooks at the end of the worm are four in number: two large, and two very small between them. These latter are present in some of the types described, but are absent in others and may have been overlooked by some authors. It is also possible that we have here a new subspecies peculiar to the Far East; but this can only be settled by comparison with the types of *Octocotyle scombri* Kuhn from Europe.' A footnote after this sentence runs: '*Octocotyle maximus*, of which there is no description available, is also from the mackerel.'

This unfortunate reference to *O. maximus* creates a *nomen nudum*, for there is no other mention of the name in the literature; and it is almost certain that he had a faulty recollection of Goto's *O. major*, for apparently (see pp. 103–4) he did not have Goto's works for reference. It is possible that he found a large specimen of *Kuhnia minor* such as those described in the present paper from Plymouth, to which he might have applied the name *Octocotyle maximus*.

Layman's figures of *Kuhnia scombri* (Figs. 2, 3), appear to be very crude, especially that of the clamp, which is over-schematized, but in a general way they resemble *K. scombri* sufficiently to be regarded as the same species. Goto's material came from somewhat farther south, off the Japanese coast, than Layman's which was from Peter the Great Bay, Vladivostok.

Specimen 'C' is becoming sexually mature at a much earlier stage than the British *K. scombri*; this may be due to local influences of temperature, etc., accelerating development, for it appears to be at the beginning of the first female phase, since ova are present in both descending and ascending parts of the oviduct. Goto figures about eighteen rounded testes, more numerous, he says, than in *K. scombri*. In both 'B' and 'A' the testes were spent but only about ten vesicular masses could be counted—about the same number as in *K. scombri*. In 'B' the skein-like appearance of the testes is shown, similar to that found at the end of a male phase in the other species, and in 'A' (probably nearing the end of the last male phase) the vitelline duct is seen distended with large droplets of a highly refringent substance from the degenerating vitellaria. Analogous specimens of *K. scombri* over 6 mm. long were found on the same fish. The wide unbranched ends of the intestine extend to the limit of the body between the nearly parallel rows of clamps.

The genital armature is very similar to that of *K. scombri* except that there is a greater difference in size between the two types of hooks (Fig. 3*n*). The figure is taken from specimen 'A' and gives the dorsal aspect of the genital bulb so that the hooks are seen from beneath: the antero-lateral pair measure 20·8 μ (blade 13·3 μ and base 7·8 μ). The inner rows of hooks are in opposite groups, converging near the middle of the aperture, the blades (7·6–7·8 μ) are more slender and acutely pointed, and the bases (5·7–4·0 μ) are more bulbous and not set at so great an angle with the blade as in *K. scombri*: their total length varied from 10·8 to 12·5 μ. Their number is probably eight on each side, though in the second 'A' specimen one hook was missing on one side, and in the other gaps in one row showed that two hooks had been lost. In specimen 'B' there are eight pairs, though they are much smaller and contracted together so that this has not been figured. Goto mentions only five pairs of hooks in this species—twelve in all. No eggs have been found.

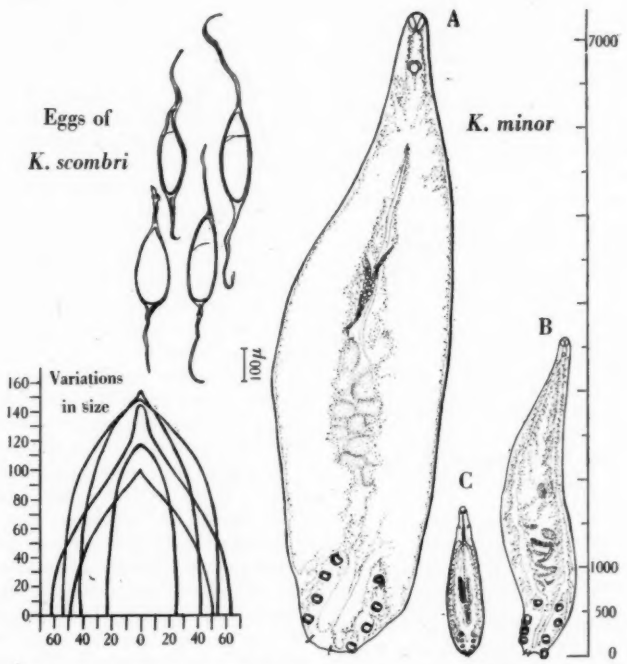

Fig. 4. *K. scombri*. Left, above: eggs, the two upper from spring, and the two lower from autumn specimens. Left, below: superimposed camera-lucida drawings of outlines of half-eggs to show variations in size and shape. *K. minor*. Right: the three known specimens of *K. minor* drawn to the same scale—'A' from off-shore mackerel landed at Plymouth (9 April 1940); 'B' from inshore mackerel at Plymouth (29 October 1940); 'C' from Goto's original figure of *K. minor* from *Scomber japonicus*, Japan. ('A', 'B', dorsal and 'C' ventral views.)

Kuhnia macracantha (Meserve, 1938) n.comb.

This species was described by Meserve (1938, pp. 48–50, Pl. 6, figs. 25–29), from an unidentified mackerel from Galapagos Islands, as *Mazocraës macracanthum*. He points out that it is closely allied to *Octocotyle minor* Goto; hence it is here transferred to the genus *Kuhnia*. It is probably quite justifiable to compare the measurements with Goto's specimen(s) since the size range is similar. *K. macracantha* ranges from 1·47 to 2·34 mm. long and 0·356 to 0·474 mm. wide. Unlike *K. minor*, the body is slightly constricted in front of the haptor and the intestinal rami and vitellaria are not present below the level of the first pair of clamps, and though the anchors are shaped like those of *K. scombri* they are relatively larger and are solid, the small hooks are *f*-shaped. The clamps are inadequately figured, but appear to resemble those of *K. minor*, being wider than long, and their relative size is about midway between those of the two previous species. The genital armature is almost exactly like that of *K. minor*, both in form and proportions of the two types of hooks (cf. Meserve, Fig. 29), but there are only four pairs of inner hooks compared with eight in *K. minor*. The ovary is said to be on the left of the mid-line, the opposite side to that which is usual in this family, though cases of *situs inversus* do occur (see Fig. 1*f*). There are ten polygonal or transversely oval testes in the same relative position as in the other species. No eggs were found in any specimens, though they were apparently mature adults.

HETERAUXESIS* IN *KUHNIA SCOMBRI*

In view of the marked heterogonic growth of those parts which appear to be of greatest diagnostic value in the genus *Kuhnia*, it was thought that a quantitative expression of their potentialities of growth might be of assistance in defining the specific differences. The growth behaviour of *K. scombri* has been studied in some detail by analysing the measurements of six entities on 148 specimens ranging from the earliest larva known to the largest adult. From this data it was found that, by the graphical application of the allometry formula, an approximation to the differential growth coefficients of the various parts to the whole could be compared for different stages in development.*

Since 'growth is concerned essentially with the multiplication of living substance', and 'on the logarithmic scale, equal spaces on the graph denote equal amounts of multiplication, whereas on the ordinary absolute scale they denote equal additions' (Huxley, 1932, p. 11); the values have been plotted on the logarithmic scale and the growth coefficients obtained from the slope of the curves.

In order to test the application of the allometry equation to the growth of these animals, a series of measurements of the haptor length and body length was plotted as logarithms on an ordinary graph (see Fig. 3): for convenience the ordinates represent the ratio of the haptor to the body as a whole and the abscissae the corresponding body lengths. All measurements in these studies have been made on mounted specimens fixed under standardized conditions in order to reduce errors due to contraction of such soft-bodied forms to a minimum: the considerable degree of scatter seen in Fig. 4 is largely due to such errors, but when the lengths were grouped (starting with those up to 1·8, 1·8–2·8, 2·8–3·8 mm., etc.) and the mean values taken (marked by crosses in the figure), a nearly straight line is obtained.†

The triangles on the same graph represent the values for specimens 'A' and 'B' of *K. minor*, and they are sufficiently removed from the other values to be taken as lying on a different curve.

In Fig. 6 the heterauxesis data for *K. scombri* have been summarized; the abscissae are the same for each entity and represent the logarithms of the mean lengths of the whole worm for each millimetre length group, and the ordinates the logarithms of the mean measurements of the five entities compared. The ordinates have not all been plotted on the same scale, but each scale has been expanded, roughly in inverse proportion to the absolute size of the entities measured: in this way the relative amount of growth is more easily compared, whereas in Fig. 7 all the curves have been drawn to the same scale so that the growth of the parts can be seen in true perspective. Both the y and x scales are in microns, and these y/x curves are represented by dots.

An alternative method of comparison is included on the same graph: the ordinates on the right represent the logs of the ratios of the four entities to the corresponding size of the haptor, the haptor being chosen as a datum for comparison owing to

* This term was proposed by Huxley, Needham & Lerner (1941) to denote the relation of growth rate of a part of a developing organism to the whole or another part, when organisms of the same group (species) but different ontogenetic stages are compared, and *allomorphosis* when similar ontogenetic stages are compared in different groups. *Allometry* being used as a general covering term.

* The simple allometry equation used is $y = bx^k$, where x is the total body length, y the size of the heterogonic part, b a constant depending on the units of measurement, and k the coefficient of differential growth.

† From the allometry equation $\log y = \log b + k \log x$, the graph of which is a straight line.

its being less influenced by contraction than the other regions of the body. The curves for these mean values are plotted with crosses.

The following are a few of the new facts brought to light by this simple mathematical arrangement of the measurement data, and which without it might have passed unnoticed, for they are not obvious from an inspection of the series of specimens.

In all the heterauxesis curves there is a sagging or decrease in slope after length group '2'. This retardation in the rate of growth corresponds to the onset of the active reproduction period (cf. Fig. 2), so that, as might be expected, the multiplication of body substance is sacrificed to some extent to the development of reproductive cells. Similarly, the recovery of the rate of growth, or its acceleration, in the second half of life may be due to a relief from the reproductive drain: this is partly explained by corresponds to the period when growth is markedly accelerated in the width of the body.

From Fig. 6 it can be seen that the clamps and anchors both may be under the same growth influence as the haptor, so that the ratio curves are approximately parallel. As these two skeletal structures are laid down very early in life and thereafter grow slowly (the k values for neither ever reaching 0·5*), their form and absolute size are of great value in specific diagnosis. The early establishment of the adult form of the haptor and its skeletal structures has been well demonstrated for one member of this superfamily (Diclidophoroidea) in Remley's description of the life cycle of *Microcotyle spinicirrus*, where the anchors are shown to be present on the haptor of the larva immediately on hatching, and the first clamps which are formed are nearly the same size as those formed later in life. Also recently, Brinkmann

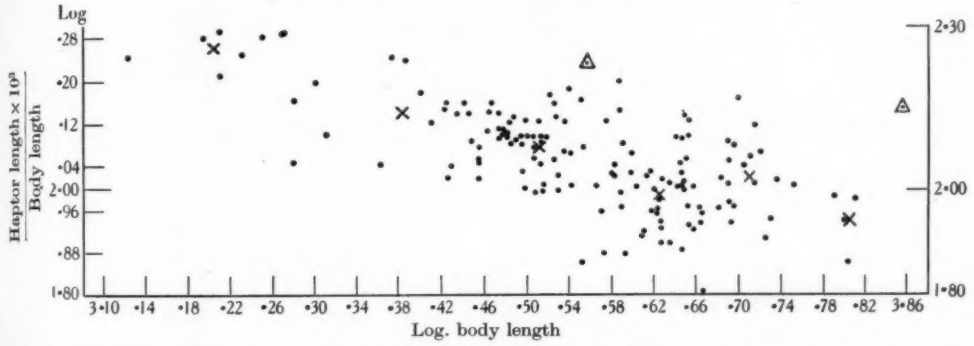

Fig. 5. Graph showing that the growth of the haptor with respect to the body as a whole obeys the log/log rule: the dots represent the actual values, and the crosses the means of the 6 mm. length groups; the triangles represent the values for specimens 'A' and 'B' of *K. minor*, showing that they lie on a different curve (cf. Fig. 7).

the majority of large forms being found in the early part of the year, so that these have survived the winter when it is likely that the activity of the gonads is reduced and growth of the body as a whole is favoured. [*Note.* From Fig. 2 it can be seen that the highest frequencies of worms with ripe gonads occur in length-group '2' and again, in '4'–'5'.]

The growth factors for the width are by far the highest ($k = 1·72$, 1·01, 1·10, 1·86, 0·80, between the six stages arbitrarily chosen); the growth is thus variable, and, as the ratio curve shows, the proportion of width to length can be of little value in diagnosis except for medium-sized worms. The haptor, on the other hand, appears to have grown very rapidly in early larval life, and in this series its increase is slow ($k = 0·4$, 0·5, 0·49, 1·0, 0·14): there is a recovery phase between stages '4' and '5' when growth is isogonic with the body length, and this (1940, p. 15) has shown that the anchors of very young specimens of *Linguadactyla molvae* (Gyrodactyloidea) are nearly the same size as those of the adults with a body nearly five times larger.

The growth of the oral suckers can be seen to have no relation to that of the haptor and its organs, but they appear to come under the same influence as the width of the body and they both share the marked acceleration between stages '1' and '2' (the k values for the oral suckers are: 1·16, 0·4, 0·3, 0·34, 0·47).

ALLOMORPHOSIS IN THE GENUS *KUHNIA*

In order to compare the dimensional and growth characters of *Kuhnia scombri* with other members of the genus, the summarized values have been

* The apparent resorption of the anchors between stages '5' and '6' cannot be explained.

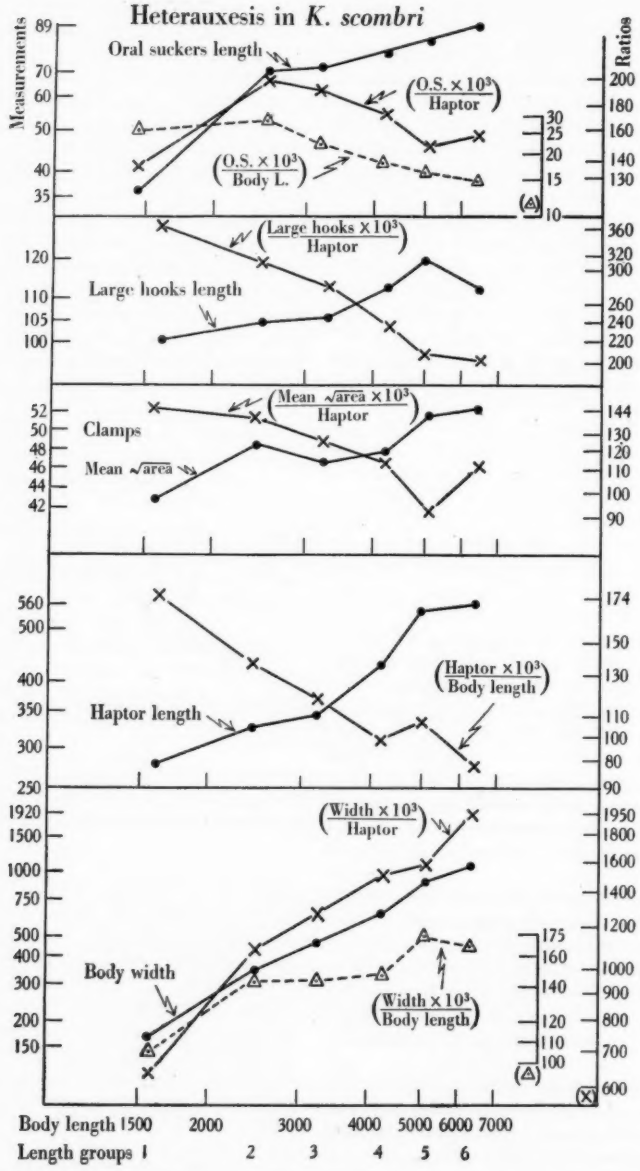
Heterauxesis in K. scombri
Measurements
Ratios
Oral suckers length
(O.S. × 10³ / Haptor)
(O.S. × 10³ / Body L.)
(Large hooks × 10³ / Haptor)
Large hooks length
(Mean √area × 10³ / Haptor)
Clamps
Mean √area
(Haptor × 10³ / Body length)
Haptor length
(Width × 10³ / Haptor)
Body width
(Width × 10³ / Body length)
Body length 1500 2000 3000 4000 5000 6000 7000
Length groups 1 2 3 4 5 6

Fig. 6. Log/log comparison of relative growth of parts of *K. scombri*: abscissae represent total body lengths; ordinates (left), the sizes of parts—curves with dots—and (right) ratios of parts to haptor, etc. All are means of grouped values—in μ (see text).

superimposed on the same y/x double log graph in Fig. 7, together with the values from the three specimens of *K. minor*, and the few data given by Meserve (1938) for *K. macracantha*. It is fortunate that the three specimens of *K. minor* are so well dispersed over the length range, so that reasonably valid comparisons may be made with the other species.

Though the haptor of *K. minor* is not separated from the body, its rate of growth is very different from that of the body as a whole: in the first half of life it is about three times that of *K. scombri* ($k = 1{\cdot}38$ as compared with $0{\cdot}44$); this causes the originally relatively, and absolutely, smaller haptor to overtake that of *K. scombri*, and its ratio to the body length increases from 13 to 17 %, whereas in

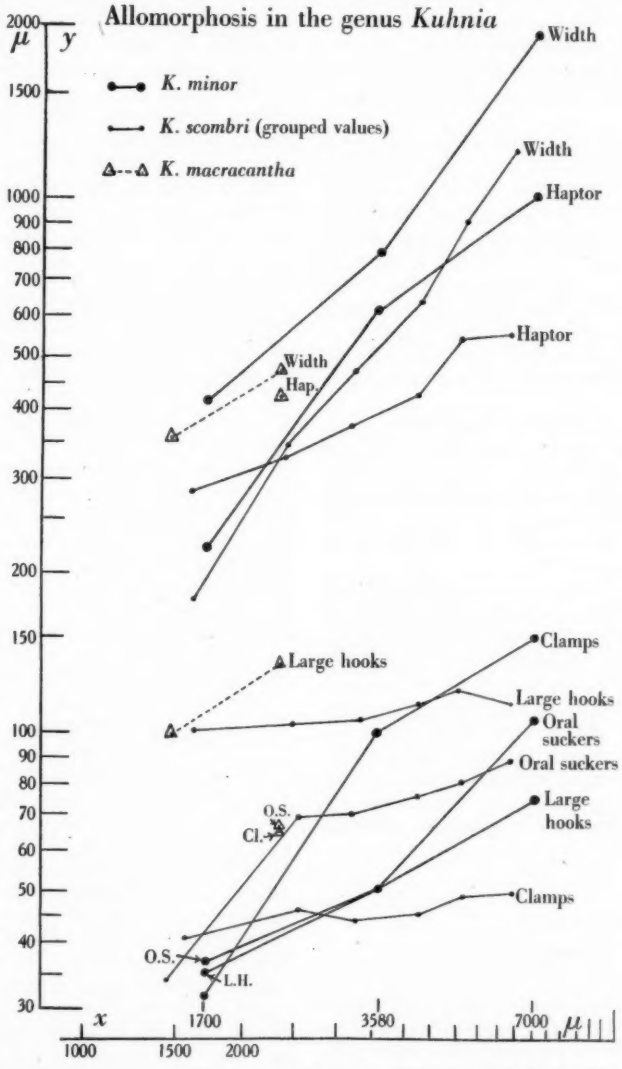

Fig. 7. Log/log comparison of relative growth of parts of species of the genus *Kuhnia*. Abscissae total length of worms and ordinates size of parts. All in μ, and for *K. scombri*—small dots—means of grouped values (see text).

K. scombri over the same periods it falls from 17 to 12%. In the second half of life the values of k for the two species are 0·72 and 0·63 respectively, and the ratio of the haptor to body falls in both (to 14 and 9%). In *K. macracantha* the characters of the haptor appear to be similar to those of *K. minor*, but its ratio to the whole is greater than at any stage in the other species: about 20 to 25%. Hence, the ratio of the haptor to the body is of limited use in diagnosis.

In *K. minor* the growth of the clamps is active throughout life and they are nearly isogonic with the haptor (for the first and second halves of life $k = 1{\cdot}46$ and 0·6); in 'C' the clamps are absolutely smaller than in *K. scombri* group '1', but in 'B' they become more than twice the size of those in *K. scombri*, and in 'A' nearly thrice. In *K. macracantha* they are even larger than in the corresponding stage of *K. minor*. Thus, since the rate of growth of the clamps in this genus seems to be a specific character, their absolute size by itself is of no value in diagnosis.

The large hooks, or anchors, on the other hand, are of such different absolute and relative sizes in the three species that they are of first importance in diagnosis. It is interesting to note that the scanty information available seems to show that in both *K. minor* and *K. macracantha* the anchors grow appreciably during adult life.

The value of studying the growth curves whenever possible in trematodes is here stressed. When the values of k are known for certain size ranges, a graph can be constructed and the curves extended so as to test the inclusion of any doubtful species which may be found outside the known size range. It is probable that without the application of this reasoning the present large specimens of *K. minor* would have been accepted as new species. It is also felt that graphical representation of this kind is of help in the understanding of possible variations within a genus.

SYSTEMATICS

Kuhnia n.g.

Synonyms: *Octostoma* Kuhn, 1829, partim (preoccupied Otto, 1823); *Octobothrium* Leuckart, 1827, partim; *Octocotyle* Diesing, 1850, partim; *Octoplectanum* Dies., 1858, partim; *Octocotyle* Dies., sensu Goto, 1894; *Mazocraës* Hermann, 1782, partim.

Generic definition. Mazocraeidae, lacking a vagina. Common genital pore with longitudinal opening guarded by a row of inwardly curved hooks on each side. Genital bulb with two antero-lateral reniform appendages, each bearing a distinctive hook with a strong outwardly curved blade, at the base of which are two broad pointed spines. At least two pairs of terminal hooks: an outer pair of anchors with a spur near mid-length, and an inner pair of smaller, twisted sigmoid hooks. In addition, the larval hooklets may persist, one pair occurring between the anchors and small hooks. Parasites on the gills of Scombridae. Other characters as in *Mazocraës* Hermann.

Type species. Kuhnia scombri (Kuhn, 1829) n.comb.

Other species. K. minor (Goto, 1894) n.comb. (Syn. *Octocotyle minor* Goto, 1894), *K. macracantha* (Meserve, 1938) n.comb. (Syn. *Mazocraës macracanthum* Meserve, 1938). (See tabular comparison, p. 190).

DISCUSSION

Goto (1894, 1898) applied the generic name *Octocotyle* Dies. to the trematode described by Kuhn (1829, pp. 357, 361–2) as *Octostoma scombri* from the gills of mackerel from East Atlantic waters; but in doing so he admits that the absence of a vagina and other characters—which he does not clearly state (1894, pp. 123, 202–3)—separate it from *Octocotyle lanceolata* (Leuck. 1827), and that it must be placed in a different genus. He does not make any nomenclatural alterations, however, other than excluding *O. lanceolata* from *Octocotyle* Dies.; but this was inadmissible because *O. lanceolata* is the type species of *Octocotyle*; accordingly its use for the trematodes from the mackerel is also inadmissible. It is obvious that Goto was justified in separating these species from *O. lanceolata* and its allies from clupeoids, though the name *Octocotyle* Dies. is a synonym which cannot be revived since its type species *O. lanceolata* (Leuck.) is synonymous with *Mazocraës alosae* Hermann, 1782, by which it is antedated. Recent authors have therefore substituted Goto's *O. scombri* by *Mazocraës scombri* (Kuhn) without inquiring into its relationships with the genotype. Its separation from the genus *Mazocraës* (along with *Octocotyle minor* Goto and *Mazocraës macracanthum* Meserve) is based on the lack of a vagina, the longitudinal disposition of the genital opening and hooks, and the presence of the reniform lobes on the genital bulb with their distinctive hooks, which are absent in *Mazocraës*. A dorsal vagina is present in the latter genus, but the genital hooks are in two transverse rows, and the species occur on the gills of Clupeidae. The generic name is proposed in honour of the discoverer of the type species.

Kuhnia is perhaps more closely related to *Mazocraeoides* Price, 1936 (Syn. *Pseudoctocotyle* Yamaguti, 1938), for, like *Kuhnia minor*, both *Mazocraeoides georgei* Price and *M. dorosomatis* (Yamaguti) (Sproston, 1945, Fig. 88*a–c*) have the haptor continuous with the body, and the clamps attached in the region of the intestinal rami and

vitellaria. *Mazocraeoides* possesses a dorsal vagina and the testes are said to be in a single lobed mass. There is also a difference in the genital hooks, for the inner ones are either very numerous or suppressed, and the outer pair enormously developed.

The genus *Grubea* Dies., 1858, resembles *Kuhnia* in other ways; it is represented only by *G. cochlear* Dies. (Sproston, 1945, Fig. 85*a–g*), found for the first time by Grube in 1885, on the gills of *Scomber scombrus* in the Mediterranean, on the same fish with *Kuhnia scombri*. It has since been recorded from the same region several times (and also on *Scomber colias*), though it has been found nowhere else. It differs from *Kuhnia* in having only one side of the haptor developed, so that there are four typical clamps on the one side and only a rudimentary one on the other. It has close relationships also with *Mazocraës* (but the presence of a vagina is not mentioned), in that the genital hooks and pore are transverse and there are two dissimilar hooks at either end of the rows very similar to those of *Kuhnia*. These four genera are thus very closely related and constitute the family Mazocraeidae.

The genus *Neomazocraes* Price, 1943, created for *Discocotyle dorosomatis* Yamaguti, 1938, is most interesting, for it appears to be transitory between Mazocraeidae and Discocotylidae (see Sproston, 1945, Fig. 89*a–d*). Price (1943, p. 10) removes it from the original genus mainly on the basis of the structure of the clamps and the genital armature (the latter being similar to *Mazocraeoides dorosomatis* from the same host, and even more like that of *Kuhnia* spp.), though it differs from all other mazocraeids in having very conspicuous **Y**-shaped vaginae opening on the margins by two large sucker-like pores, a feature so characteristic of *Discocotyle* Dies. In view of the redescription of the clamps of *Kuhnia* (and their structure is similar in all other mazocraeids), the suggestion of Price that *Neomazocraes* should be included in the same family must be accepted with reserve—unless a re-examination of the type material shows a clamp structure very different from that depicted by Yamaguti (1938, Pl. 3, fig. 9). The clamps are shown with poorly cuticularized dorsal and ventral loops, both of which are split in the median line, as in Discocotylidae; the middle piece has some resemblance, though remote, to that of mazocraeids, but it lacks the typical scoop-shaped form of the latter.

A full list of the synonyms of the species of *Kuhnia* and other genera is given in the systematic work cited (Sproston, 1945).

SUMMARY

A new genus, *Kuhnia* n.g., is created for the mazocraeidid monogenetic trematodes from the gills of mackerel. The type species, *K. scombri* (Kuhn), is redescribed (also *K. minor* (Goto), from the first specimens to be found outside Japanese waters), special attention being paid to the cuticularized parts, and the clamp skeleton is newly interpreted.

An alternation of sex phases has been found in Monogenea, which is well marked in *K. scombri*, resulting in changes in appearance which are described.

The comparison of heterauxesis curves constructed from the summarized data from six sets of dimensions shows that retardations in the rates of growth may be correlated with the onset of maximal reproductive activity, and accelerations with the relief from reproductive drain. Localized growth influence (similar shaped curves, with low post-larval coefficients of growth) has been found in the haptoral region, which is in marked contrast to the growth picture of the cuticularized oral suckers and the width of the body—the latter having both the highest and most variable growth coefficients between the six arbitrary length groups compared. Thus, neither absolute measurements nor ratios are, by themselves, of any value in specific diagnosis.

Graphical representation of allomorphosis for the genus *Kuhnia* affords a synoptic view of the changes brought about by the different types of differential growth of similar organs in each species throughout post-larval life. The dissimilar growth coefficients for certain dimensions are utilized in specific diagnosis. The relative diagnostic value of different entities is examined: the eggs of *K. scombri* are shown to be highly variable in size and shape.

The new facts obtained from the application of this metrical treatment, and its further application as a test for conspecificity of specimens outside the known size range, are discussed.

The systematics of the family Mazocraeidae are reviewed, and emended diagnoses of three species of *Kuhnia* are given in tabular form.

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Note. A summary of the numerical data on which the graphs have been based is being deposited in the archives of the British Museum (Nat. Hist.) in accordance with the recommendations of the British Association's Committee on Biological Measurements.

SUMMARY OF SPECIFIC DIAGNOSES IN THE GENUS *KUHNIA*

	K. scombri (Kuhn)	*K. minor* (Goto)	*K. macracantha* (Meserve)
Body:	Widest near the middle and tapering to a narrow anterior region and behind, to just in front of the heart-shaped haptor, which is set off from the body. (Larval forms with nearly parallel-sided body narrower than haptor: up to about 2 mm. long. Other data for larvae in brackets below)	Tapering more abruptly in anterior fifth and widest region extends farther back than middle; tapering slightly to a truncate hind end which includes haptor, which is thus not set off from body, and it contains intestinal rami and vitellaria	Almost parallel-sided: tapering very slightly in front, and only slightly constricted in front of the haptor which may be wider than body in front. Haptor is bluntly tapered and not clearly set off from body
Length (mm.):	(1·32) 1·8 3·6 6·5	1·7 3·58 7·0	1·473 2·340
Width (mm.):	(0·13) 0·23 0·54 1·10	0·418 0·786 1·92	0·356 0·474
% of length	(10) 12 15 17	24 22 27	24 20
Haptor (mm.):	(0·23) 0·34 0·49 0·625	0·222 0·618 1·00	About 420
% of length	(17) 12·5 10 9	13 17 14	More than 20 %
Growth factor (*k*)	0·44 and 0·63	1·38 and 0·72	?
Clamps:	In two divergent rows, first pair midway between mid-line and edge of haptor, fourth pair on edge about a third to a fourth length of haptor from the end. Nearly square or slightly longer than wide. (Structure as in *Mazocraës*)	In two parallel rows, first pair midway between mid-line and edge of body, fourth pair on hind edge of body. They are wider than long with thicker sclerites, especially in larger worms	In two slightly converging rows near to the margins of haptor. Slightly wider than long. Structure as in *K. scombri* though distal part of tendon appears very heavily sclerosed. Sclerites moderately thick
Mean $\sqrt{area}$ (μ)	(35) 42 46 52	33 99 148	Mean about 65
% *of haptor*	(17·5) 14 12 9 or 10	15 16 15	15·5 % of haptor length
Growth:	Relatively slow, always <0·5	*k*=1·46 and 0·6	?
Anchors:	Stout, with strong hollow hook and ridged shaft. Spur at about mid-length in small worms to three-fifths from proximal end in large ones	Slender and wholly solid with thin U-shaped hook and spur about mid-length. The shaft is ridged	Stout, but wholly solid, otherwise shaped like those of *K. scombri* though hook less curved. Spur two-thirds from proximal end
Length (μ):	(100) 102 105 113	37 53 75	100 132
% *of haptor:*	(44) 36–30 26 20	17 8·5 7·5	24 31 of haptor
	k=less than 0·1 and 0·3	*k*=0·49 and 0·51	*k* greater than 0·6
Small hooks:	Sigmoid with twisted shaft and sickle-like free end	Sigmoid, twisted pot-hook shaped	Sigmoid with sickle-shaped hook and small spur two-thirds from reflexed proximal end
Length (μ):	(11·5) 13 17 22	Shaft about 9	20
Oral suckers	(36) 56 75 90	38 53 106	Relatively large
(mean length μ)	*k*=(1·16) 0·4 9·3 0·5	*k*=0·43 and 1·03	
Intestine:	Unbranched crura only in haptor: extend to between third and fourth clamp usually unequal in length.	Unbranched ends of crura between clamps to tip of body. Rami lateral to clamps to end of body. Some vitellaria in haptor	Crura extend unbranched into haptor to last pair of clamps, usually unequal in length and rather narrow. No intestinal rami or vitellaria in the haptor
	No vitellaria in haptor nor for its own length in front of it		
Genital armature:	Inner hooks: five in each row each about 17 μ long with semicircular blade sharply reflexed on shaft Lateral hooks: broad blades, total length about 21·5 μ Hook ratio, 4:5 in length	Inner hooks: eight in each row converging slightly near the middle. 10–12·5 μ long, blades more slender, less curved and reflexed than in *K. scombri* Lateral hooks: 21 μ long Hook ratio: 4:7 or 8	Inner hooks: four in each row with slightly curved blade and bulbous base Lateral hooks: as in other species but markedly larger than inner ones Ratio apparently about 1:2
Ovary:	On right of median line	On right of median line	On left of median line
Eggs:	Elongate ellipsoids, operculate with a filament at each pole: anterior about egg length, posterior shorter. Variable shape and size 312–194 μ long and 126–49 μ wide (extreme measurements not corresponding)	Eggs unknown	Eggs unknown
Hosts:	*Scomber scombrus* L., Skagerrak, North Sea, English Channel, East and West Atlantic, Mediterranean. *S. colias* from Mediterranean. *S. japonicus* (Houttuyn) at Vladivostok and Misaki (Japan)	*S. japonicus* (Houttuyn) from Hagi and Misaki, Japan. *S. scombrus* L., west English Channel area	From the gills of an unidentified mackerel taken at Tagus Cove, Albemarle Island, Galapagos Islands

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A NOTE ON THE COMPARATIVE ANATOMY OF THE CLAMPS IN THE SUPERFAMILY DICLIDOPHOROIDEA (TREMATODA: MONOGENEA)

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(With 1 Figure in the Text)

A re-examination of the haptoral sclerites forming the clamps, or 'armoured suckers', in Diclidophoroidea shows that they can be grouped into four main types, representing lines of evolution from what is here regarded as the primitive, or more generalized, form of clamp, that of the family Mazocraeidae. It has been shown that the form of the clamp skeleton must be regarded as of primary importance in the classification of the superfamily, since not only is it correlated with certain diagnostic characters of the soft parts of these worms, but it is usually fully developed some time before these parts make their appearance. Due attention has not always been paid to the detail of the clamps in systematic works, so that the position of some forms must remain uncertain until they can be redescribed. Owing to the sclerites often being twisted bars, curving through three dimensions, with the primary bars often jointed or fused, the appearance of different types of clamp is at first sight highly complex. The difficulty of interpreting them is increased by their being semi-transparent, and appearing of different shapes when viewed from various angles. These obscurities disappear when they are regarded as modifications of the fundamental plan found in *Mazocraës* and its allies.

Goto (1894, pp. 20–5) was the first to treat the anatomy of the 'posterior suckers' in detail, though he did not recognize a common plan in the various forms he described. Bonham & Guberlet (1937, pp. 282–3) substantiated Goto's conclusion that the clamps are formed from a condensation in the prismatic fibres which form the basis of the 'sucker'. Goto found that the solubility of the sclerites in potash solutions precluded a chitinous nature (1894, footnote 2, p. 20), and Remley (1942) applied Campbell's (1929) chitin test and found it negative —both for the clamps and other cuticular structures, of *Microcotyle spinicirrus*. These cuticular structures are best seen after fixation with Bouin-Duboscq fluid, which stains them a bright yellow: they do not take other stains readily, except when slightly decomposed. Fig. 1 is a semi-diagrammatic illustration of seven types of sclerite development, and is intended to show the homologies among the parts: to facilitate this they have all been drawn from the ventral side, and slightly tilted in the same direction from the horizontal plane, so that the dorsal sclerites can be seen behind the ventral. In *Hexostoma*, *Choricotyle* and *Diclidophora* the clamps have a lateral asymetry, so that they have all been taken from the morphological left side of the worms, i.e. the left side of each diagram is the nearer to the median line of the worm. The main muscles are represented by dotted lines.

In Mazocraeidae alone, the fundamental plan of cuticularization appears to be developed in its most straightforward form. These clamps have hitherto been described as formed of two U-shaped bars joined anteriorly by a cross-bar; Goto and subsequent authors have perpetuated this error. There is, in fact, a continuous flattened band, twisted as it is bent upon itself, to form two more or less rectangular loops: movable on the spring-like hinges between them (see *Mazocraës*, Fig. 1). A third loop—a U-shaped, middle, opposable piece—lies between them, its ends articulating beneath the shoulder springs. It has a thin scoop-like expansion attached to the U-shaped rim, directed nearly at right angles to the ventral and dorsal loops when the clamp is closed upon itself (see figures of a disarticulated clamp of *Kuhnia scombri*, in Sproston, 1945*a*, Fig. 3 *j–l*). The 'cross-bar' is really the optical section of the anterior wall of the very thin cuticular clamp-capsule. The tendon of the main retractor muscle, running from the median line of the haptor, is only lightly cuticularized in Mazocraeidae, and is applied along the dorsal wall of the capsule, attached to the middle of the dorsal loop as it enters (perforates) the clamp capsule, and crosses its cavity to be threaded through a triangular hole in the 'middle piece' where it expands in a broad, more or less T-shaped, piece—acting as a sliding catch. In the majority of the Diclidophoroidea the tendon is more heavily cuticularized, particularly in the dorsal and anterior regions; it forms what some authors call the 'middle piece' or T-piece, though the latter usually refers only to the expanded end, so that to avoid ambiguity it is better to refer to the whole structure as the *tendon*, since it is the site for attachment of the main muscles. It is noteworthy that in Mazocraeidae, the spring action tending to open the clamp when the muscle is relaxed is located in the shoulders between the

dorsal and ventral loops; but in these other groups the more highly developed tendon, arching over the median line of the capsule, acts as the spring (see figure).

In the family Discocotylidae several modifications occur in the clamp skeleton: in *Chimaericola* Brinkmann (1942), there are apparently three springs—the dorsal and ventral loops seem to have been reduced so that only the two shoulder regions remain, while the central arched sclerite is clearly homologous with the tendon—this may represent a transition form from the lateral spring type of the

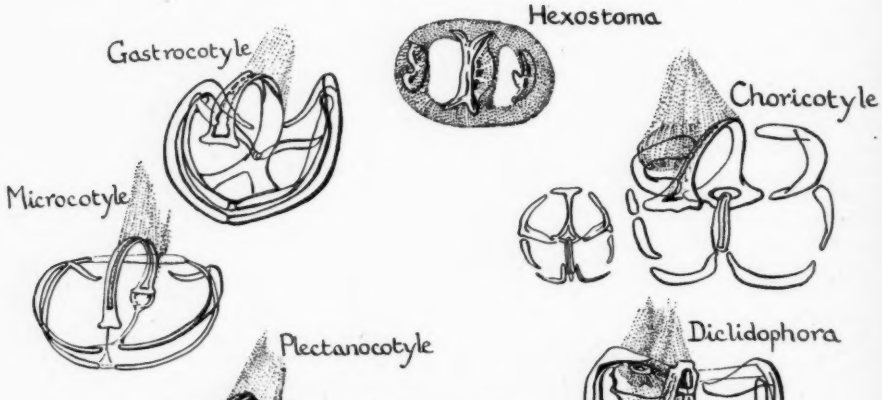

Fig. 1. Types of clamp development in Diclidophoroidea: showing a probable evolutionary sequence from the unbroken double loop in *Mazocraës*, through the discocotylid types on the left (*Plectanocotyle*, *Microcotyle* and *Gastrocotyle*), where the sclerites have become jointed but are still symmetrical, to the diclidophorid types on the right (*Diclidophora* and *Choricotyle*) where the tendency to develop a sucker has brought about asymmetry. Finally, in *Hexostoma*, there is an extreme reduction to three asymmetrical and irregular sclerites supporting the muscles of a sucker bounded only by prismatic fibres.

Mazocraeidae to the median spring plan of the greater number of forms existing to-day. The more typical discocotylid clamp is similar to that of *Microcotyle*, but stouter. There is usually a tendency for the rami of the middle, opposable, loop to be jointed about half way along their length, and in practically all species both the middle and ventral loops are divided in the median line; also in all, except the older specimens of *Plectanocotyle gurnardi* (see Fig. 1), the dorsal loop has become reduced to two spurs lying in the anterior region of the capsule. In Plectanocotylinae there are additional cuticularizations: rib-like bars are developed in the ventral wall of the capsule, while the opposable piece retains the scoop-like plaque through which is threaded the tendon (cf. *Mazocraës*). In *Plectanocotyle*, however, the tendon is fixed to the rim of the plaque by narrow extensions of the arms of the T-shaped end, though there is still a certain amount of freedom in the sliding joint—particularly in younger worms. In other subfamilies the opposable, middle loop has no such plaque and its connexion with the tendon is not cuticularized. In the subfamily Anthocotylinae, while the structure of the clamps is quite typically discocotylid, their great inequality of development in *Anthocotyle* is unique: the three posterior pairs are minute with very slender sclerites (the clamps are also all poorly developed in *Tagia* and *Hemitagia* [=*Heterobothrium* spp. of Meserve, 1938]), and in this respect, and several others, they suggest a transition between Discocotylidae and Microcotylidae.

In Microcotylidae there is a very marked weakening in the sclerites (see *Microcotyle*, Fig. 1): the tenuous connexions between the rami of the ventral and middle loops are sometimes difficult to

trace, and the articulation of the middle loop with the shoulders of the ventral loop and its reduced dorsal spurs is only partial, because it is usually fused with the anterior wall of the capsule ('cross-bar' of the previous authors). The poor development of the clamp sclerites in this family may be correlated with the very large number of clamps present, their number being continually added to, even in adult life. Specific differences in Microcotylinae are concerned partly with the form of the ventral end of the tendon, and of an accessory trifid or dagger-shaped piece sometimes attached to the dorsal end of the tendon. In Gastrocotylinae the reduction of the sclerites has not gone so far, and indeed, in their additional sclerites, they are a counterpart of Plectanocotylinae in the family Discocotylidae. In *Gastrocotyle* the dorsal end of the tendon is expanded and its bifurcated ends are attached to the two rami of a well-developed opposable piece; the dorsal wall of the capsule is supported by two oblique bars (these may represent the anterior rim of the opposable piece—cf. *Plectanocotyle*—but in *Gastrocotyle* they have become separated from it and lie dorsally to the tendon). In *Thoracocotyle* several such transverse bars are said to be present, apparently both in the dorsal and ventral walls of the capsule.

In *Diclidophora* the four clamps on each side of the haptor are very powerfully developed, and the mazocraeid plan has been further modified, via the discocotylid: an asymmetry has developed owing to the attachment of more than half the main muscles directly on to the inner moiety of the dorsal loop (see Fig. 1). The clamps on either side the worm are mirror images of one another. The middle, or opposable piece, (?) plus the dorsal part of the tendon, has been split longitudinally; it remains in the dorsal region of the capsule and shares some of the muscles with the arched, ventral, part of the tendon. This latter is hollow and shows numbers of small projections along its central ridge to which muscle fibres are attached; its anterior end is fused with a rectangular ring (? part of the middle loop), and into this, often irregular, structure, the double median sclerite articulates. The rami of the opposable piece are completely jointed at the sides in all members of this family. This longitudinal splitting of the opposable piece (? plus dorsal region of the tendon) can be visualized as evolving through a condition like that in *Gastrocotyle*: supposing the tendon to be more heavily cuticularized, the bifurcation to be continued forwards, and a more intimate union with the rami of the opposable piece effected posteriorly.

In the figure of *Diclidophora* the two halves of the ventral loop have been artificially separated so that the 'opposable piece' can be seen more clearly; in life the distal ends both articulate with the end of the tendon. The spur-like remnant of the dorsal loop on both sides is widely flanged; on the inner side (left of figure) the flange is very wide and extends to the articulation of the tendon, in some species fusing with the ring-like structure. The greater part of the muscles of the clamp of Diclidophoridae are attached to this inner remnant of the dorsal loop, and here they form an incipient sucker in *Diclidophora*, further developed in Choricotylinae to form a true sucker. In several species of *Diclidophora* this muscle bundle is peculiar in that it appears to have a small internal tendon in the form of a cuticularized ring or disk: Cerfontaine (1898, pp. 307–11) first drew attention to this structure. Probably, as a counteraction to the strong pull on the inner half of the clamp, the Diclidophorinae develop cuticular irregularities on the ventral face of the outer half of the clamp; thus such a friction plate would react on the gill lamella adjacent to that clasped by the clamp and make the hold more effective. In order to avoid confusion in the figure, these cuticular structures have been omitted: in *D. denticulata* they are short broad spines, in *D. lusci* cobblestone-like elevations, in *D. merlangi* and in *Choricotyle* spp., dovetailing ridges and rows of minute bars respectively.

In *Choricotyle* and its allies, probably owing to the special development of the muscular sucker, the clamps are kept in an open position, i.e. the muscles on the arched tendon are less powerful than in other groups, since most of them are diverted to the sucker, and the spring is incompletely compensated so that it maintains the open position of the clamp, causing the whole apparatus to act more like a reinforced sucker than a clamp. Owing to the strain on the parts, occasioned by this permanently open position, some of them have become disarticulated; but the new orientation provides a firm framework to the rounded concave sucker, the homologies of which can be seen from Fig. 1. The main diagram of *Choricotyle* is adapted from Llewellyn's excellent figures (1941, p. 401, Figs. 6–7) of *C. chrysophryi*, and the smaller one to the left from Goto's *Cyclobothrium sessile* (1894, Pl. 10, fig. 5); the others are original.

In *Hexostoma* it appears that the end-point has been reached both in development of the sucker and in the reduction of the sclerites. The adhesive unit is asymmetrical like those of the Diclidophoridae, but the sclerites have fused on either side and in the middle into highly irregular pieces, each acting as a tendon for the retractor muscles. They have become deeply imbedded in the prismatic fibres which form the marginal ring and the median partition. The homology here is, of course, entirely supposititious; nor is this fusion and reduction of sclerites conceived as taking place at the same evolutionary level as that in the (?) more primitive

Chimaericolidae, though these two types of clamp present an interesting instance of convergence.

From the foregoing considerations it is thought that it is far more satisfactory to use the form of the clamp skeleton as a basis for classification rather than the number of sclerites composing it (as advocated by Price, 1936, pp. 11–12); or, as the older authors have done, the number of clamps on the haptor, and whether these are stalked or sessile, taking no cognizance of the form or relative orientation of the component parts. With these criteria in mind a revised classification of the Diclidophoroidea has been prepared (see Sproston, 1945*b* for keys, definitions and illustrations of the forms referred to in this paper); but in view of the suggested homologies of the clamps in this superfamily it was thought timely to introduce the following descriptive terms and to indicate their significance from the standpoint of comparative anatomy. The most generalized clamp occurs in the Mazocraeidae where it consists of three U-shaped loops: a continuous *dorsal* and *ventral loop*, the shoulders between which form *springs* tending to open the clamp capsule; and a *middle loop* or *opposable piece* (articulating beneath the shoulder-springs) connected with a weakly cuticularized tendon carrying the main retractor muscles, opposing the springs and tending to close the clamp. These primary sclerites undergo jointing and some reduction in Discocotylidae and Microcotylidae, and a further asymmetrical development in Diclidophoridae: in which families the more heavily cuticularized tendon now forms a median arched spring. In Hexostomatidae the sclerites show an extreme reduction to three asymmetrical pieces, imbedded in a mass of prismatic fibres of the capsule wall: this acts not as a clamp, but as a reinforced sucker, a function foreshadowed in Choricotylinae (Diclidophoridae).

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A NEW SPOROZOON, *GREGARINA RHYPAROBIAE* N.SP., FROM A TROPICAL COCKROACH, *RHYPAROBIA MADERAE*

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(With 16 Figures in the Text)

CONTENTS

	PAGE
Introduction	195
Methods	195
General description	195
Conclusions	198
Summary	198
References	198

INTRODUCTION

The gregarine described in this paper was found in the alimentary tract of a tropical cockroach collected near Entebbe, Uganda, in 1927–8, by Dr C. A. Hoare. This cockroach was subsequently identified by Dr B. P. Uvarov, of the Imperial Institute of Entomology, as *Rhyparobia maderae*.

The material was obtained from five cockroaches and consisted of: (1) the entire alimentary tract of host specimen no. 2 containing gregarines in the midgut; (2) two pieces of midgut with hepatic caeca attached from host specimen no. 5 partly with the contents, consisting of semi-digested food, removed; (3) a number of individual sporonts of the gregarine isolated from the midgut of host specimen no. 4; (4) smears of the gut contents of host specimens nos. 1 and 3.

Watson (1916), in her survey of the eugregarines from the Myriapoda, Coleoptera and Orthoptera of the world, does not refer to any species of *Rhyparobia* as a host, nor does Foerster (1938 *a*, *b*) in his more recent work.

An extensive search of the literature has revealed only one brief reference to gregarines parasitic in *R. maderae*, by Hegner & Chu (1930), who state that their material was obtained from Manila and remark that '*Rhyporobia* [sic] *maderae*, of which two specimens were examined, also contained many gregarines of several species'.

METHODS

All the available material had been fixed in alcoholic Bouin's fluid and subsequently preserved in 70% alcohol. The isolated specimens were stained in borax carmine and mounted entire. The alimentary tracts were divided into suitable lengths and serial sections cut from them which were stained in Mayer's haemalum.

Drawings of all the complete specimens (eighty in number) and of selected sections were made with Abbe's camera lucida for purposes of measurements and comparison. The system of axes and ratios used in making the measurements and statistical comparisons was that adopted by Henry (1938).

GENERAL DESCRIPTION

The material available consisted of a considerable number of sporonts, both single and in association, and serial sections of the complete mid- and hindgut of host specimens nos. 2 and 5 in which were found various stages in the development of the trophozoite and a single specimen each of a sporozoite and a gametocyst.

Sporozoite. No loose sporozoites were observed in the lumen of the gut, but a single specimen was observed penetrating a host cell (Fig. 1). This sporozoite was about 48 μ long, sharply pointed anteriorly and rounded posteriorly. The nucleus, which lies in the posterior half of the body, is about 6 μ in diameter and consists of a homogeneous mass of fairly evenly distributed chromatin.

Trophozoite. Trophozoites of various stages could be seen in the sections, attached to cells of the wall of the intestine (Fig. 2). The epimerite is deeply embedded in the host cell, which is generally much hypertrophied; the protomerite occupies a depression of the intestinal surface, the deutomerite projecting freely into the lumen (Fig. 2).

The mature trophozoite just prior to detachment from the host cell measured about 400 μ. The epimerite, which forms approximately one-tenth of the total length of the trophozoite, is a swollen

ellipsoidal structure borne upon a broad cone arising from the apex of the protomerite; its exterior is grooved, so that in tangential longitudinal section it has an appearance of root-like processes (see Figs. 6 and 8–13), easily mistaken for an epimerite of *Actinocephalus*. With regard to the interior of the epimerite, the ellipsoidal apex has a honeycomb-like structure, while the basal cone is solid but with a concave base (see Fig. 5). The protomerite is rounded and measures about one-seventh of the total length of the trophozoite; its anterior edge becomes considerably thickened to form a clear cap, the apex of which merges smoothly into the base of the epimerite. There is a conspicuous septum between the protomerite and the deutomerite marked externally by a deep constriction. The epicyte is thick in both divisions of the body, and the endoplasm appears to be of the same consistency in both. The deutomerite is peculiar in having a distinct keel or ridge which runs along one side and is a constant feature in transverse sections; it is even more prominent in the sporont (see Fig. 14). The nucleus shows a fine uniform granulation with from one to six deeply staining endosomes. It is usually situated in the anterior half of the deutomerite (see Fig. 2).

Sporont. The epimerite becomes separated from the protomerite either just prior to or just after the detachment of the sporont from the host cell. It breaks off across the apex of the cone, forming an irregular fracture which becomes ultimately smooth (see Figs. 3, 4).

The mature sporont reaches a mean length of 557 μ, but the largest observed specimen was 765 μ long. The ratio of the length of the protomerite to the total length is 1 : 1·6, of the width of the protomerite to the width of the deutomerite is 1 : 1·6, and the ratio of the greatest width to length of the mature sporont is 1 : 2·4.

Association. The sporonts are bi-associative, and associations range in length from 810 to 1230 μ, with a mean length of 940 μ. The two associates are firmly united.

The primite does not differ markedly in form from the solitary free sporont, but the protomerite is somewhat longer in proportion to the total length, and the clear cap at the anterior edge has become rounded. The mean length of the observed specimens was 460 μ. The ratio of the length of the protomerite to the total length is 1 : 5·3, of the width of the protomerite to the width of deutomerite is 1 : 1·5, and the ratio of greatest width to total length is 1 : 2·6. The posterior end of the deutomerite is somewhat flattened.

The satellite is very similar to the primite, but shows some modification of the protomerite which is markedly compressed anteroposteriorly and of which the anterior cap is flatter, of more uniform thickness, and shows a conspicuous double recess into which fits the flattened posterior end of the deutomerite of the primite. The mean length of the observed specimens was 480 μ, and it is thus clear that there is some slight compression of the body in individuals in syzygy. The ratio of the length of the protomerite to the total length is 1 : 7·3, of the width of the protomerite to the width of the deutomerite is 1 : 1·4, and the ratio of greatest width to length is 1 : 2·8 (see Fig. 7).

Table 1

Dimensions (in μ)

	Loose sporonts	Primites	Satellites	Range
Epimerite	55	—	—	—
Length of protomerite	83	87	66	35–150
Length of deutomerite	474	373	414	260–665
Width of protomerite	150	115	119	80–240
Width of deutomerite	233	176	172	120–345
Total length	557	460	480	335–765

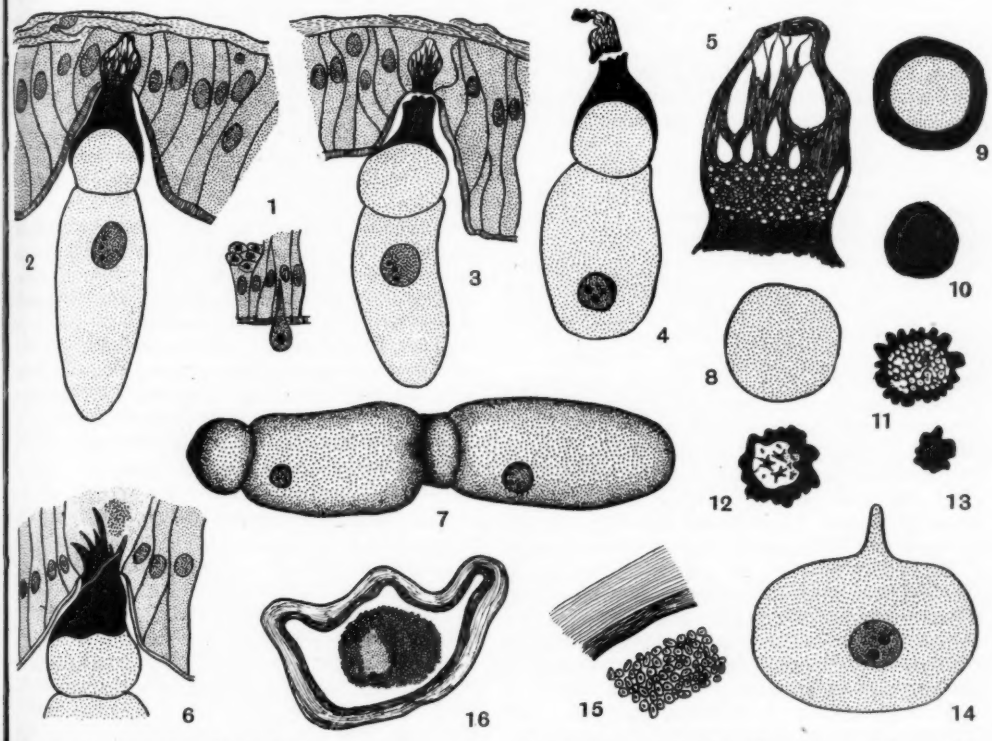
Proportions

	Loose sporonts	Primites	Satellites
Length of protomerite/total length	1 : 6·7	1 : 5·3	1 : 7·3
Width of protomerite/width of deutomerite	1 : 1·6	1 : 1·5	1 : 1·4
Greatest width/total length	1 : 2·4	1 : 2·6	1 : 2·8

The dimensions and proportions of the sporonts, both solitary and in association are summarized in Table 1.

Cyst and Spores. Only one specimen of a cyst was observed in the material available, this occurring in a series of transverse sections of the hindgut (see Fig. 15). The cyst had a mean diameter of 230 μ and was of markedly irregular form, with a thick wall of fibrous appearance 20 μ thick. This consisted of three distinct layers—a thin deeply stained inner lining membrane, a thicker moderately stained fibrous layer, and an outer still thicker lightly stained fibrous layer (see Fig. 16). The whole of this wall is no doubt formed by the sticky, gelatinous,

transparent secretion of the associated sporonts. The innermost layer formed from the residual protoplasm and showing radial striations described by Weschenfelder (1938) for *Actinocephalus parvus* bodies was observed outside the cyst, hence the distorted form of the cyst and the wide gap between its inner wall and its contents were in all probability artefacts due to faulty fixation or sec-



Figs. 1–16. *Gregarina rhyparobiae* n.sp. Structure of sporozoite, trophozoite and sporont as seen in transverse sections of the intestine of the host.

Fig. 1. Sporozoite securing attachment to cell of intestinal wall of host (*circa* × 100).
Fig. 2. Mature trophozoite attached to intestinal wall of host (*circa* × 100).
Fig. 3. Sporont breaking away from intestinal wall of host. Note fracture plane of epimerite (*circa* × 100).
Fig. 4. Sporont loose in lumen of gut with attached epimerite (freed from intestinal wall) lying close to it (*circa* × 100).
Fig. 5. Median longitudinal section of epimerite to show honeycomb-like internal structure (*circa* × 500).
Fig. 6. Tangential longitudinal section of epimerite, showing appearance of rootlike processes (*circa* × 100). This also shows hypertrophy of host cell with disintegration of nucleus.
Fig. 7. Association, from a whole mount, showing mode of attachment of satellite to primite (*circa* × 75).
Figs. 8–13. Serial transverse sections through the epimerite. Note especially the external grooving in Figs. 11, 12 and 13, and the internal honeycomb-like structure in Figs. 11 and 12 (*circa* × 200).
Fig. 14. Transverse section through the deutomerite to show the characteristic keel (*circa* × 200).
Fig. 15. Transverse section through a cyst, showing gametes and residual cytoplasm (*circa* × 100).
Fig. 16. Portion of cyst wall and gametes under higher magnification to show the three layers of the wall (*circa* × 500).

is not seen. Within the cyst was an almost spherical mass of small rounded or ellipsoidal cells, each with a conspicuous ellipsoidal nucleus; these were evidently gametes or zygotes. Although the cyst wall was intact, a considerable aggregation of these tion cutting. According to Watson (1916) spores can develop and be expelled within the intestine of the host, possibly resulting in reinfection, but no spores were observed in any of the material available in this case.

CONCLUSIONS

The septate primite and satellite, the simple, more or less globular form of the epimerite and the association of the sporonts in pairs lead to the conclusion that the sporozoon described above belongs to the genus *Gregarina*. As no gregarine has previously been described from this host, and it seems to be generally accepted that gregarines are host specific, it may be concluded that this represents a new form for which I propose the name *Gregarina rhyparobiae* n.sp.

Four points alone call for special comment. In the first place, the anterior end of the sporozoite penetrating the host cell appears to be more elongate and sharply pointed than is usual for members of the genus *Gregarina*, judging by the figures and descriptions of Lühe (1904), Léger & Duboscq (1902 and 1904) and Watson (1916); but, on the other hand, the sporozoite is an exceedingly active organism, and its shape in sections may well depend very largely on its posture at the moment of fixation. Furthermore, the structure of the trophozoites and sporonts present in the material makes it clear that they all belong to the same species, and that no member of the genera *Pyxinia* or *Stylorhynchus*, which frequently have such narrow, pointed sporozoites, was present. In the second place, the external grooving and internal honeycomb-like structure of the epimerite have not been referred to in descriptions of species of this genus as far as I have been able to discover, by previous authors, but they do not detract from the essentially simple character of the organ. The resemblance to the epimerite of *Pyxinoides* is striking, but in the descriptions of many of the species of *Gregarina*, especially those published in recent years, the epimerite is not clearly shown in figures and its detailed structure is therefore obscure. Epimerites of similar general shape are, however, found in *G. macrocephala* Schneider Labbé, *G. davini* Léger & Duboscq and *G. marteli* Léger, but whether these show external grooving and internal lattice work as in *G. rhyparobiae* is not clear. In the third place, the distinct keel along the deutomerite appears to be a unique structure which, considered in conjunction with the distinctive epimerite and sporozoite, may be regarded as sufficient to justify the establishment of a new species on morphological grounds as well as on grounds of host specificity.

In the fourth place the irregular form of the cyst is peculiar, but is almost certainly an artefact, since in no other member of the Gregarinidea is such an irregular gametocyst formed.

In conclusion, it may be noted again that Hegner and Chu (1930) mention the finding of 'many gregarines of several species' in the same host. However, since these authors had obviously not made a detailed study of their material, it may be doubted if more than one species (possibly the one described in this paper) was present. There is, however, the possibility that tangential or transverse sections of the epimerite of *G. rhyparobiae* were seen by these authors and mistaken for the epimerite of *Actinocephalus*.

SUMMARY

A new species of cephaline gregarine, *Gregarina rhyparobiae* n.sp., parasitic in the gut of a tropical cockroach *Rhyparobia maderae*, from East Africa, is described.

I take this opportunity to acknowledge my indebtedness to Dr Hoare for placing the material at my disposal, and for guiding and criticizing the work recorded above. I am also deeply indebted to Dr C. M. Wenyon, F.R.S., Director-in-Chief, and the authorities of the Wellcome Foundation for so generously placing their admirable facilities at my disposal.

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THE NEMATODE LUNGWORMS (SUBORDER STRONGYLINA) OF NORTH AMERICAN DEER OF THE GENUS *ODOCOILEUS*

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(With 11 Figures in the Text)

I. INTRODUCTION

North American deer of the genus *Odocoileus* Rafinesque, 1832, can probably all be placed in two species: *O. virginianus* (Boddaert), the white-tailed deer, and *O. hemionus* (Rafinesque), the black-tailed and mule deer.* The nematode lungworms of certain of the subspecies of these two species have been investigated by various parasitologists in the last thirteen years, and some previously known forms recorded and new forms described from them. I have had the opportunity to study specimens of most of the lungworm species reported from *Odocoileus*. The present paper is a review of previously published information and a record of new observations.

The nematode lungworms of ruminants are strongylines which fall into two main groups, one consisting of the genus *Dictyocaulus* Railliet & Henry, 1907, and the other represented by the genus *Protostrongylus* Kamenskiĭ, 1905. *Dictyocaulus* may be placed in a family of its own, Dictyocaulidae Skriabin, 1941. *Protostrongylus* and allied genera constitute within the family Metastrongylidae Leiper, 1909, a group which may be regarded as a subfamily Protostrongylinae Kamenskiĭ, 1905. The protostrongyline lungworms have been placed in a number of genera which cannot be individually treated here. A complete review of the genera and species of the subfamily is in preparation. The genera already established which I recognize as valid are eight in number, as follows: *Protostrongylus* Kamenskiĭ, 1905 (syn. *Synthetocaulus* Railliet & Henry, 1907); *Spiculocaulus* Shul'ts, Orlov & Kutas, 1933; *Cystocaulus* Shul'ts, Orlov & Kutas, 1933; *Neostrongylus* Gebauer, 1932; *Elaphostrongylus* Cameron, 1931; *Varestrongylus* Bhalerao, 1932; *Pneumostrongylus* Mönnig, 1932; and *Muellerius* Cameron, 1927.

A recent paper by Dikmans (1939) contains a record of all adequately identified lungworm species found in *Odocoileus* up to 1939. Dikmans records these parasites from three species of *Odocoileus*, of which, however, *O. columbianus* (Richardson) has been shown by Cowan (1936) to be a synonym of *O. hemionus*. Since 1939 no new species of lungworms has been described from these hosts, but the results of important work by Frans C. Goble on the pathology of lungworm infection in *O. virginianus borealis* Miller appeared in 1941, and more recently records by the same investigator (Goble, 1943) and by Cheatum (1942) of lungworm infection in *O. v. virginianus* (Boddaert) have been published.

Through the kindness of several parasitologists I have been able to study the following known species of lungworms recovered from species of *Odocoileus*: *Dictyocaulus viviparus* (Bloch, 1782) Railliet & Henry, 1907; *Elaphostrongylus odocoilei* Hobmaier & Hobmaier, 1934; *Protostrongylus macrotis* Dikmans, 1931; and the form known currently as *Pneumostrongylus alpenae* Dikmans, 1935.* I have also received from E. L. Cheatum of Delmar, New York, a single male of an apparently new species from the common white-tailed or Virginia deer (*Odocoileus virginianus virginianus*).

II. THE LUNGWORMS OF *ODOCOILEUS VIRGINIANUS* (BODDAERT, 1784)

Dikmans (1935*a*) reported the first lungworms from *O. virginianus*; these he identified as *Dictyocaulus filaria* (Rudolphi, 1809) Railliet & Henry, 1907, *D. viviparus*, *Protostrongylus* sp., and *Pneumostrongylus* sp. They were recorded as occurring in the 'lungs', but were not localized more precisely.

* For this view I am indebted to Dr Remington Kellogg (*in litt.*), United States National Museum, Washington, D.C.

* Thanks are due to Dr M. A. Stewart, Division of Entomology and Parasitology, University of California, Berkeley, Dr C. M. Herman, California Division of Fish and Game, San Francisco, and Dr F. C. Goble, Game Research Center, Delmar, New York, for specimens of *D. viviparus*; Dr Michael Hobmaier, George Williams Hooper Foundation for Medical Research, University of California Medical School, San Francisco, for specimens of *E. odocoilei*; Dr G. Dikmans, Zoological Division, U.S. Bureau of Animal Industry, for specimens of *P. macrotis*; and E. L. Cheatum, Game Research Center at Delmar, for specimens of *P. alpenae*.

In a later paper of the same year Dikmans (1935*b*) described the last two lungworms as new species—*Protostrongylus coburni* and *Pneumostrongylus alpenae*—and gave as their type host *Odocoileus virginianus*, i.e. the northern white-tailed deer (*O. v. borealis*) of Michigan. The following year O'Roke (1936) discussed further problems relating to the lungworm parasites of the same subspecies of host.

Goble (1941) conducted a study of the changes in the lungs of the northern white-tailed deer caused by lungworms considered by him to be *Dictyocaulus viviparus* and *Protostrongylus coburni*. Recently he (Goble, 1943) has reported the incidence of adults supposedly of the latter species in the common white-tailed deer (*Odocoileus v. virginianus*) of New York. The identification of *Protostrongylus coburni* was in all cases, however, made on the basis of larval morphology and of the presence of adult worms in sections of infected lung tissue.

Cheatum (1942) has recently established the occurrence of *Dictyocaulus viviparus* in the bronchi and bronchioles of *Odocoileus v. virginianus* in New York.

In February 1943, Mr E. L. Cheatum sent me through Dr Goble a single male lungworm which the latter had not had time to identify before beginning a period of several months in the armed services, and which had been recovered from a white-tailed deer, in the course of life history work being conducted at Delmar, New York, by Cheatum. It has proved to belong to a new species which I consider to fall into the genus *Pneumostrongylus*. Because of its significance to the lungworm problem in the white-tailed deer and because of the favourable opportunities that I have had to compare it with other protostrongylines, description at this time based on a single specimen seems warranted. I therefore propose to call it *Pneumostrongylus tenuis*, n.sp.

Pneumostrongylus tenuis n.sp.
(Figs. 1, 2 and 3*a*)

Diagnosis. Pneumostrongylus. Male: length 45 mm.; maximum width 0·180 mm.; oesophagus, length 0·650 mm., width 0·080 mm.; ventral rays subequal; lateral rays of about same length; externodorsal ray longer and thicker; dorsal ray hemispherical, with three long, ray-like and two smaller papillae; gubernaculum 0·110 mm. long, with weakly sclerotized corpus and more strongly sclerotized crura (corpus 0·100 mm. long, crura 0·023 mm. long); spicules 0·195 mm. long, with two strongly developed striated alae and a longitudinal slit in the first part of the distal half of the lamina. Female unknown; larvae unknown.

Type host. Common white-tailed or Virginia deer, *Odocoileus v. virginianus* (Boddaert).

Habitat. Small bronchiole of lung.

Type. Male, U.S. Nat. Mus. Helm. Coll. No. 45103.

Pneumostrongylus tenuis differs from the type species of its genus—*P. calcaratus* Mönnig, 1932, from the Impala (*Aepyceros melampus*)—in a number of points. The most important appear to me to be the following: shorter spicules and gubernacular crura (0·195 and 0·023 mm. long respectively, as compared with 0·384–0·421 mm. and 0·056–0·060 mm. respectively for *P. calcaratus*); a dorsal ray relatively much larger in comparison with the other rays; and a bursa which is not divided into two lateral lobes as in *P. calcaratus* (Fig. 3). It may be noted that the number and distribution of papillae on the dorsal ray are the same in both species.

The addition of a new species to *Pneumostrongylus* raises several points in regard to the status of certain other lungworm species in ruminants. The single male of *P. tenuis* was associated with larvae which Cheatum at first thought probably belonged to the same species. Subsequent discoveries have not borne this out; for, some time after sending me the male of *P. tenuis*, he discovered a second entire male lungworm, a posterior end of a third male, and a posterior end of a female, all in the lungs of *Odocoileus v. virginianus* and associated with the same type of larvae. The two posterior ends were forwarded to me, and the male proved not to belong to *P. tenuis*, but to represent the form known as *P. alpenae*; the female appeared from Dikmans's description to be a member of the same species; and Cheatum also identified the entire male as *P. alpenae*. Dikmans (1935*b*) noted certain significant differences in spicular, gubernacular and dorsal ray morphology between *P. alpenae* and *P. calcaratus*, but his interpretation of the parts of the gubernaculum in the latter species was partly erroneous. He referred to the corpus of the gubernaculum as the 'gubernaculum' and to the crura as the 'unpaired accessory piece'. In describing *Protostrongylus coburni* in the same paper he referred to the crura as the 'paired accessory pieces' or 'gubernacula', and to the capitulum as the 'unpaired accessory piece'. Thus he felt the crura of *Pneumostrongylus alpenae* to be homologous with the capitulum in *Protostrongylus*. I follow here the nomenclature of Shul'ts *et al.* (1933) for the gubernacular complex in the Protostrongylinae; it is superior to that of other workers—namely, of Cameron (1927), who called the capitulum and crura 'gubernaculum' and 'telemon' [*sic*] respectively; of Gebauer (1932), who called these parts 'telamon' and 'gubernaculum' respectively; and of Dikmans (1935*b*), because in it is recognized the fact that the complex of sclerotized pieces—capitulum, corpus, and crura—largely dorsal to the spicules in a male of the *Protostrongylus* group, constitutes a single structure homologous

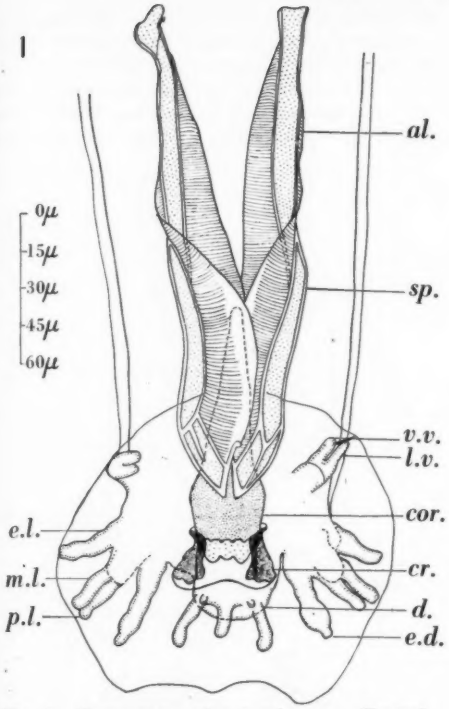


Fig. 1. *Pneumostrongylus tenuis* n.sp. Ventral view of male posterior end.

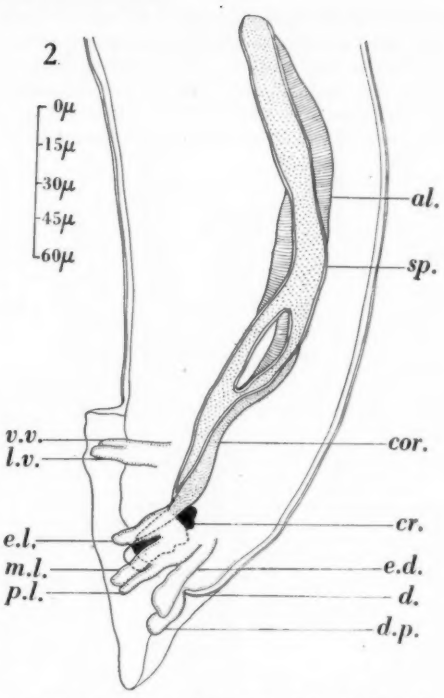


Fig. 2. *Pneumostrongylus tenuis*. Lateral view of male posterior end.

Lettering: *al.* spicular ala; *an.* anus; *bu.* bursa; *cap.* capitulum; *cor.* corpus; *cr.* crus; *d.* dorsal ray; *d.p.* dorsal ray papilla; *e.d.* externodorsal ray; *e.l.* externolateral ray; *gub.* gubernaculum; *l.v.* lateroventral ray; *m.l.* mediolateral ray; *p.* pes; *ph.* phasmid; *p.l.* posterolateral ray; *pv.* provagina; *scl.* sclerotized ornamentations; *sp.* spicule; *vu.* vulva; *v.u.* vagina uterina; *v.v.* ventroventral ray.

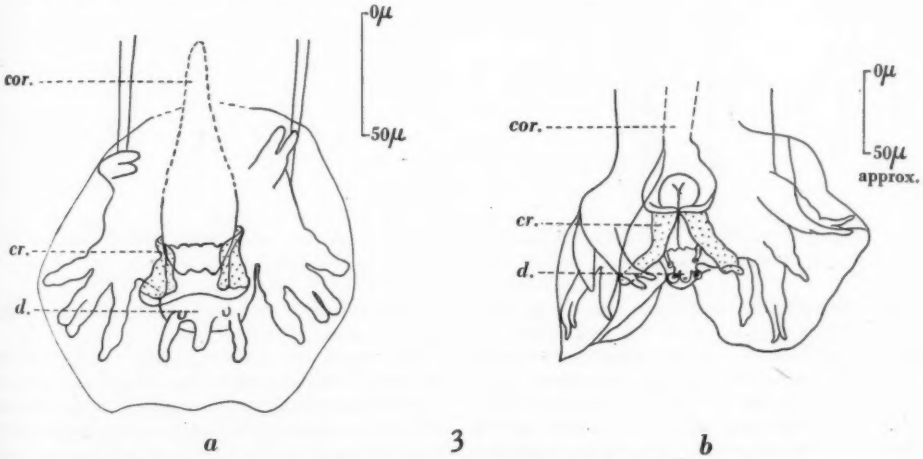
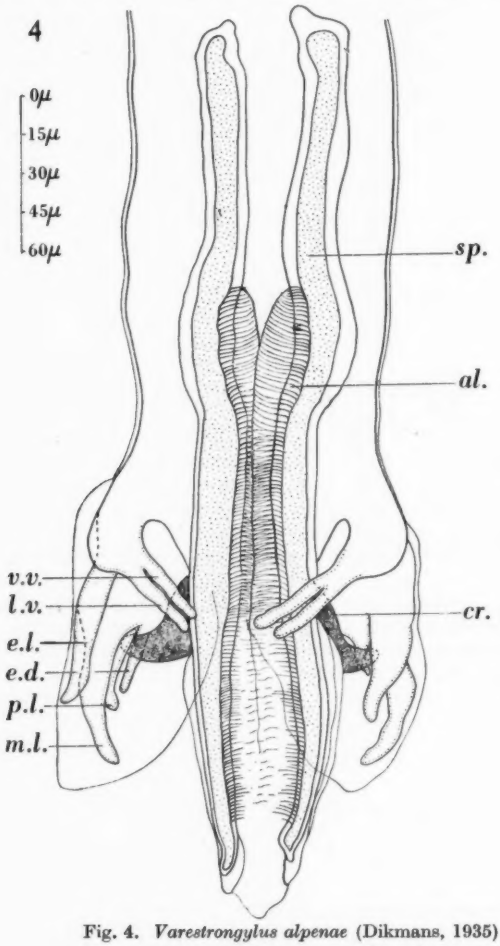


Fig. 3. Bursae of species of *Pneumostrongylus*: (*a*) *P. tenuis* n.sp., (*b*) *P. calcaratus* Mönnig, 1932 (after Mönnig, 1933).

with the simple gubernaculum of most other strongylines, and the fact that the telamon in the sense of its original usage by Hall (1921) is found in some lungworms as a sclerotized piece in the ventral wall of the cloaca. Chitwood & Chitwood (1938), in their monograph on nematology, have accepted the latter had no 'gubernaculum', and regarded the presence of such a structure as characteristic of the former. Both forms, however, have equivalent parts, for what Mönnig called the 'central unpigmented part of the telamon' and what Dikmans called the 'gubernaculum' are the same thing



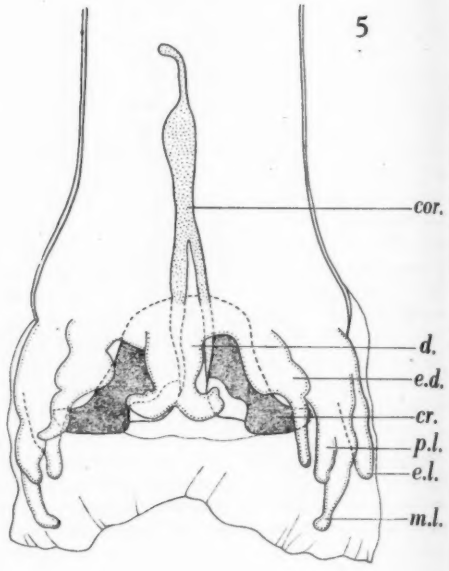


Fig. 4. *Varestrongylus alpenae* (Dikmans, 1935) n.comb. Ventral view of male posterior end.
Fig. 5. *Varestrongylus alpenae*. Dorsal view of male posterior end; spicules omitted.

Russian terminology, and I also follow it here. Mönnig (1933), who followed the terminology of Cameron (1927), described and figured the corpus of *Pneumostrongylus calcaratus* as part of the 'telamon' [crura] and stated that a 'gubernaculum' [capitulum] was lacking. The corpus in this species is apparently rather weakly sclerotized anteriorly, as in *P. tenuis*. In differentiating *P. alpenae* from *P. calcaratus* Dikmans accepted it as a fact that the [corpus]. Thus *P. alpenae* and *P. calcaratus* cannot be distinguished on this basis.

As originally described the genus *Pneumostrongylus* Mönnig, 1932 (December) shows certain resemblances to the genus *Varestrongylus* Bhalerao, 1932 (September). This led me to suspect that the former might be a synonym of the latter, but recently with the help of Dr G. Dikmans I have concluded that the two genera are best considered

distinct. I have been very fortunate in being able to study specimens of *V. pneumonicus** Bhalerao, 1932, type of *Varestrongylus* and parasitic in the Bharal, or blue wild sheep (*Pseudois nayaur*), the Hodgson argali (*Ovis ammon hodgsoni*), and two forms of the Asiatic ibex (*Capra sibirica*, which is considered by some a synonym of the European *C. ibex*)—the 'Hill' goat (probably *C. sibirica filippii*) and the 'Tibetan' goat (*C. sibirica* subsp.?). *Varestrongylus pneumonicus* is peculiar in the fact that the distal half of the corpus is almost as strongly sclerotized as the crura, and furthermore by the fact that the crura are small appendages situated some distance proximally to the distal end of the corpus. The gubernaculum of *V. pneumonicus* is illustrated in Fig. 7. In its general outlines its corpus is rather like that of a species originally described by Mueller (1890) from the German red deer (*Cervus elaphus germanicus*) as *Strongylus sagittatus* and referred to *Protostrongylus* by Cameron (1927) and later to *Cystocaulus* by Böhm & Gebauer (1934). The gubernacula of these two species are further similar in that the crura of *Strongylus sagittatus* are also

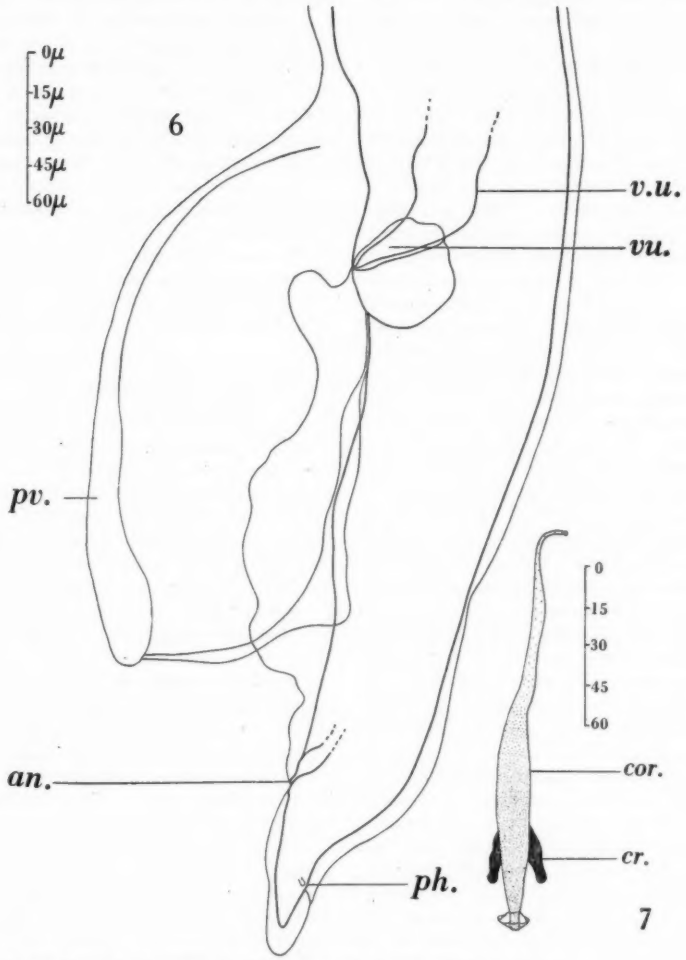


Fig. 6. *Varestrongylus alpenae*. Lateral view of female posterior end.
Fig. 7. *Varestrongylus pneumonicus* Bhalerao, 1932. Dorsal view of gubernaculum of male.

* Very kindly supplied by Dr G. D. Bhalerao, Imperial Institute of Agricultural Research, Mukteswar-Kumaun, United Provinces of Agra and Oudh, India.

attached to the corpus some distance from its end. Furthermore, the dorsal rays of both are slender and terminate in papillae—several in *Varestrongylus pneumonicus* and apparently two in *Strongylus sagittatus*. I believe that both belong to the same genus; *S. sagittatus* accordingly becomes *Varestrongylus sagittatus* (Mueller, 1890) n.comb.

Both *Pneumostrongylus calcaratus* and *P. tenuis* differ from these species in the nature of their gubernacula, the crura of which are terminal and the corpora of which are weakly sclerotized anteriorly, and in the nature of the dorsal ray, which in *Pneumostrongylus* is not slender and is much like that of *Protostrongylus*, particularly in the case of the mound-like ray in *Pneumostrongylus tenuis*. On the other hand, the form called by Dikmans (1935*b*) *P. alpenae* has a dorsal ray almost identical with that of *Varestrongylus sagittatus*. Its gubernaculum is somewhat different, however, in having terminal crura and a posteriorly split corpus. Yet the resemblances of *Pneumostrongylus alpenae* are much greater to *Varestrongylus* than to *Pneumostrongylus*, and the gubernacular character by itself does not, in my opinion, justify a separate genus. I therefore transfer this species to *Varestrongylus*, where it becomes *V. alpenae* (Dikmans, 1935) n.comb.

A species described by Stroh & Schmid (1938) from the common (?) roe deer (*Capreolus c. capreolus* [?]) as *Protostrongylus capreoli* is another of the *Pneumostrongylus-Varestrongylus* complex. Its dorsal ray appears to be very short, thick, and supplied with three terminal papillae suggestive of *Pneumostrongylus*. However, the figures of this ray are not detailed, and in the spicular, gubernacular, and bursal structure, and particularly in the form of the lateral rays, this species closely resembles *Varestrongylus alpenae*. I therefore refer it to *Varestrongylus* as *Varestrongylus capreoli* (Stroh & Schmid, 1938) n.comb.

The fact that *Pneumostrongylus tenuis* and *Varestrongylus alpenae* have been definitely identified from New York deer, whereas adult specimens of *Protostrongylus coburni* have not yet been recovered, suggests that the records of Goble (1943), reporting supposedly on the incidence of the last-named species, do not refer to this form but to one or both of the other two species. Mr E. L. Cheatum has kindly consented (*in litt.*) to my recording his observation that the larvae with which he has been working in his life-history studies have all been smaller than those described by Dikmans (1935*b*) for *P. coburni*; the former have been associated with the adults of *Varestrongylus alpenae* and were the same as those identified as *Protostrongylus coburni* by Goble (1943). Both Cheatum and Goble have concluded that *P. coburni* cannot be considered as an established parasite of *Odocoileus virginianus* in New York, and that the published records by Goble (1943) actually refer to *Varestrongylus alpenae*. Further investigation now being conducted by Cheatum and Goble will clarify this point. The earlier records of O'Roke (1936) and Goble (1941) on the occurrence of *Protostrongylus coburni* in Michigan deer should then be re-examined in the light of this newer knowledge.

P. coburni as described by Dikmans (1935*b*) bears considerable resemblance to *P. boughtoni*, as recently described by Goble and the writer (Goble & Dougherty, 1943) from varying hares (*Lepus americanus*). The two species appear to differ, however, in the structure of the gubernaculum, particularly of the capitulum, and of the externodorsal ray. Their larvae also show differences in the form of the tail. The females, however, appear to be indistinguishable.

The lungworm parasites of *Odocoileus virginianus* thus include five known species: *Dictyocaulus filaria*, *D. viviparus*, *Varestrongylus alpenae*, *Pneumostrongylus tenuis*, and *Protostrongylus coburni*.

III. THE LUNGWORMS OF *ODOCOILEUS HEMIONUS* (RAFINESQUE, 1817)

The first lungworm to be recorded from *Odocoileus hemionus* was identified by Canavan (1931) as *Synthetocaulus commutatus*. The host was given as *Mazama hemionus* from western United States; I assume from its name, however, that it must have been *Odocoileus h. hemionus*. *Synthetocaulus commutatus*, now to be known as *Protostrongylus pulmonalis* (v. Frölich, 1802) Goble & Dougherty, 1943, is, however, found only in hares in Europe, and there is no evidence to indicate that it ever occurs in ruminants there. It has never been recorded in the New World. Canavan gave the following dimensions: males, 26 mm. long; females, 37 mm. long; spicules of males 148–185 μ long. Dikmans's measurements (1931) for *P. macrotis* Dikmans, 1931, were as follows: males, 26 mm., females, 45–47 mm., and spicules 200 μ; I have seen shorter females and male specimens with somewhat shorter spicules. Thus, since Canavan's measurements fall largely into the range so far known for *P. macrotis*, I am led to believe that he probably dealt with Dikmans's species.

Dikmans (1931) described a lungworm from *Odocoileus hemionus* as *Protostrongylus macrotis*; the type specimens were recovered from the bronchi of the Rocky Mountain deer (*Odocoileus h. hemionus*). The following year he (Dikmans, 1932*a*) recorded as a new host for this species the 'black-tailed deer, *Odocoileus columbianus*', and gave Yellowstone Park, Wyoming, as the locality. Inasmuch as *O. h. columbianus* and closely allied subspecies occur naturally only on the Pacific coast of the United States and Canada, west of the Cascade and Sierra

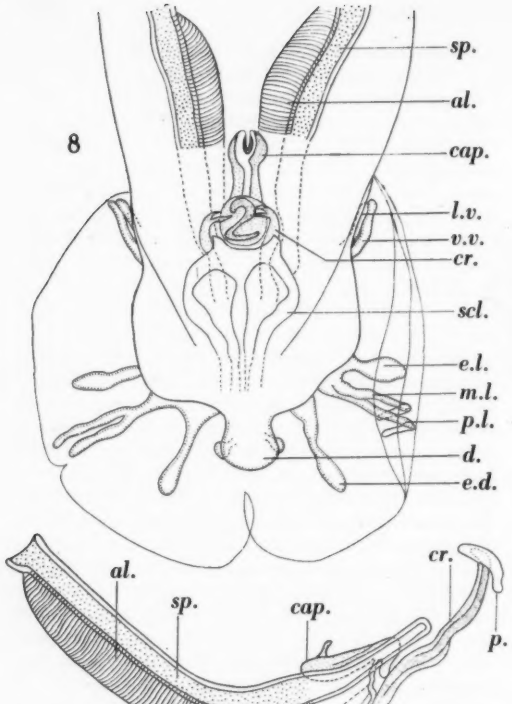
Nevada mountain ranges, I made inquiry concerning the authenticity of this host and geographical record. Dr Hadleigh Marsh, of the Veterinary Research Laboratories, Bozeman, Montana, from whom the Bureau of Animal Industry had received the specimens in question, has very kindly informed me (*in litt.*) that the host from which these lungworms were recovered was actually *O. h. hemionus*, but that through a misunderstanding based on the fact that the host had been referred to as a 'black-tail deer', without indication of the scientific name, Dr Dikmans had quite naturally assumed it to be *O. h. columbianus*. Therefore *Protostrongylus macrotis* has not yet been shown to occur in *Odocoileus h. columbianus*.

Protostrongylus macrotis is an exceptional member of the Protostrongylinae in that it is the only species definitely known to occur in more than one family of hosts. Aside from *Odocoileus h. hemionus* (family Cervidae), Dikmans (1932*b*) has recorded specimens recovered from the American pronghorn antelope (*Antilocapra a. americana*) (family Antilocapridae). European workers, especially in the early part of the present century, but even in the past ten years, have reported species such as *Protostrongylus rufescens* (Leuckart, 1865) Kamenskiĭ, 1905, and *Muellerius capillaris* (Mueller, 1890) Cameron, 1927, from a variety of ruminant hosts; but these records have been based on misidentifications. The status of *Protostrongylus macrotis* as a species of *Protostrongylus* could be challenged on the basis of Dikmans's original description, but study of specimens gives support to the view that it can be included in this genus—although admittedly as a somewhat aberrant form.

Figs. 8 and 9 illustrate the posterior end of the male in dorsal and ventral view. The arc supporting the bursa of species of *Protostrongylus* is in *P. macrotis* rather weakly sclerotized, but highly elaborate, and interconnected with sclerotizations of the adjoining part of the body proper. In the extent of its development it is somewhat similar to the complex arc found in the single known species of *Neostrongylus*—*N. linearis* (Marotel, 1913) Gebauer, 1932. In my opinion this structure, of wide occurrence in the Protostrongylinae, is deserving of a special name, and accordingly I propose to call it an *arcus* (Latin, bow). The bursal rays are characteristic enough of *Protostrongylus*; but the gubernaculum is rather specialized. Dikmans (1931), in his original description, figured it as lacking a capitulum; if correct, such a character would necessitate the removal of *P. macrotis* from *Protostrongylus*. The gubernaculum in this species is peculiarly difficult to study because of the fact that the male posterior end will clear but poorly. However, I have been able to make out a somewhat more elaborate structure than that described by Dikmans; it is illustrated in part in Figs. 8 and 9, and in entirety in Fig. 10. Its structure can be interpreted in terms of the capitulum, corpus, and crura of the gubernaculum of a typical *Protostrongylus*. The crura are quite similar in morphology to those generally found, except that they have on their distal ends small structures, rather faintly sclerotized and resembling feet; these can be conveniently termed *pedes* (Latin, feet—sing. *pes*). The corpus, however, differs rather importantly from the typical form. Instead of continuing anteriorly from the proximal ends of the crura, it doubles back postero-ventrally. As in other species of *Protostrongylus*, it is weakly sclerotized. The capitulum is unlike that of other members of the genus in that, instead of having lateral extensions, the ends of which project ventrally and posteriorly, it consists of two pigmented divisions extending anteriorly from a sclerotized base and connected for their full length by a more weakly sclerotized part which has at its anterior limit a ventrally directed, strongly sclerotized hook projecting ventrally. The base of the capitulum has a strongly sclerotized ornamentation resembling the number '2'. Although the gubernaculum of *P. macrotis* thus has certain unique characters, it does not seem profitable to erect a new genus for this species unless similar but distinct species can be found that constitute a more or less homogeneous group. It may for the time being, therefore, be regarded as an aberrant member of the genus *Protostrongylus*.

Hobmaier & Hobmaier (1934) discovered in the Columbian black-tailed deer (*Odocoileus h. columbianus*) a new species which they placed in the genus *Elaphostrongylus*. In common with *E. cervi* Cameron, 1931, *Odocoileus odocoilei* Hobmaier & Hobmaier does not, in the adult stage, occur in the lungs of the host. It is found in the lymphatic spaces of the connective tissue surrounding veins beneath the spine and their adjacent branches. Fig. 11 illustrates the male posterior end. Although it seems logical that *Elaphostrongylus cervi* and *E. odocoilei* should belong to the same genus, and although the two species are quite similar in the unusual split structure of the dorsal ray, a significant difference exists between the gubernaculum of *E. odocoilei*, as figured by me, and that of *E. cervi* as described and figured by Cameron (1931). I have observed small terminal crura at the distal end of the corpus in the former species, whereas Cameron described the gubernaculum of the latter as a simple sclerotized piece. Such a difference in structure would, in my opinion, preclude the placing of these two species in a single genus. However, specimens of *E. cervi*, when restudied, may give evidence of small crura, and it seems more reasonable to leave *E. odocoilei* in *Elaphostrongylus* pending such an investigation.

The occurrence of *Dictyocaulus viviparus* in *Odo-*



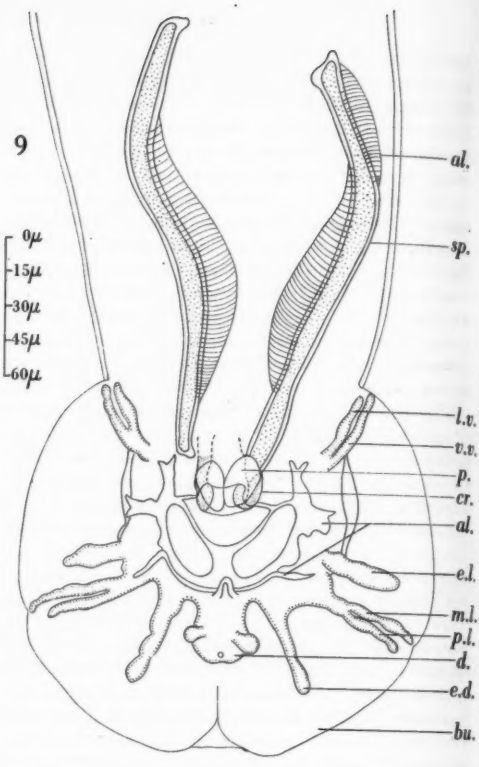
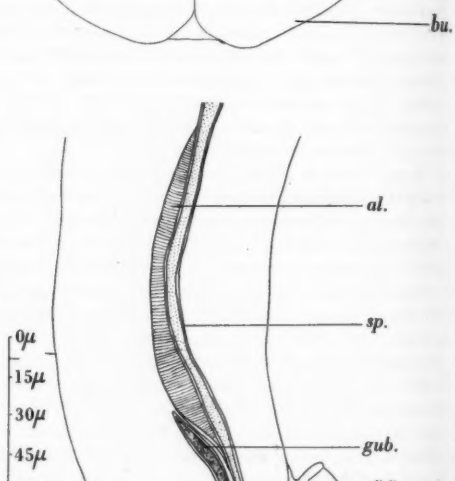


Fig. 8. *Protostrongylus macrotis* Dikmans, 1931. Dorsal view of male posterior end.

Fig. 9. *Protostrongylus macrotis*. Ventral view of male posterior end.

Fig. 10. *Protostrongylus macrotis*. Lateral view of spicule and gubernaculum of male.

Fig. 11. *Elaphostrongylus odocoilei* Hobmaier & Hobmaier, 1934. Lateral view of male posterior end.



coileus h. hemionus and of *Dictyocaulus filaria* in *Odocoileus h. columbianus* was reported by Dikmans (1936) and by Freeborn & Stewart (1937) respectively, and I have identified as *Dictyocaulus viviparus* specimens recovered from the bronchi of the latter host by Dr C. M. Herman.

The lungworm parasites of *Odocoileus hemionus* thus include four known species: *Dictyocaulus filaria, D. viviparus, Elaphostrongylus odocoilei*, and *Protostrongylus macrotis*.

IV. SUMMARY

1. Seven species of lungworms are so far known to occur in North American deer of the genus *Odocoileus*: *Dictyocaulus filaria* (Rudolphi, 1809) Railliet & Henry, 1907; *D. viviparus* (Bloch, 1782) Railliet & Henry, 1907; *Varestrongylus alpenae* (Dikmans, 1935) n.comb.; *Pneumostrongylus tenuis* n.sp.; *Elaphostrongylus odocoilei* Hobmaier & Hobmaier, 1934; *Protostrongylus coburni* Dikmans, 1935; and *P. macrotis* Dikmans, 1931. Specimens (from deer) of all these species except *Dictyocaulus filaria* and *Protostrongylus coburni* have been studied in the course of this work.

2. Two subspecies of *Odocoileus virginianus* (Boddaert) and two of *O. hemionus* (Rafinesque) have been reported as hosts for lungworms. These may be listed with their established lungworm parasites as follows:

Common white-tailed or Virginia deer, *Odocoileus v. virginianus* (Boddaert): *Dictyocaulus viviparus, Varestrongylus alpenae*—new record, *Pneumostrongylus tenuis*—new record.

Northern white-tailed deer, *Odocoileus v. borealis* Miller: *Dictyocaulus filaria, D. viviparus, Varestrongylus alpenae, Protostrongylus coburni*.

Rocky Mountain mule deer, *Odocoileus h. hemionus* (Rafinesque): *Dictyocaulus viviparus, Protostrongylus macrotis*.

Columbian black-tailed deer, *Odocoileus h. columbianus* (Richardson): *Dictyocaulus filaria, D viviparus*—new record, *Elaphostrongylus odocoilei*.

3. A new species, *Pneumostrongylus tenuis*, is based on a single male lungworm from a bronchiole of *Odocoileus v. virginianus*. *Pneumostrongylus tenuis* differs from *P. calcaratus* Mönnig, 1932, genotype of *Pneumostrongylus* Mönnig, 1932, in spicular, gubernacular, and bursal characters.

4. *Pneumostrongylus alpenae* Dikmans, 1935, is transferred to the genus *Varestrongylus* Bhalerao, 1932, as a result of the study of specimens of *Pneumostrongylus alpenae* and *Varestrongylus pneumonicus* Bhalerao, 1932, genotype of *Varestrongylus*. The former becomes *Varestrongylus alpenae* (Dikmans, 1935) n.comb. Also placed in this genus are *Varestrongylus sagittatus* (Mueller, 1890) n.comb. (syn. *Strongylus*, or *Protostrongylus sagittatus*) and *Varestrongylus capreoli* (Stroh & Schmid, 1938) n.comb. (syn. *Protostrongylus capreoli*).

5. The reports of *Protostrongylus coburni* from *Odocoileus v. virginianus* by Goble (1943) were based on larvae and adult lungworms in sections of lung tissue. Subsequent observations by Cheatum (*in litt.*) strongly indicate that these were actually *Varestrongylus alpenae*.

6. The structure of the gubernaculum in *Protostrongylus macrotis* is redescribed, and the conclusion reached that this form may be regarded as an aberrant species of *Protostrongylus*. Appendages at the distal ends of the gubernacular crura are given the name *pedes* (sing. *pes*), and the sclerotized arc, found in the bursa of a number of genera of the Protostrongylinae, the name *arcus*.

7. The fact that crura have not been described for the gubernaculum of *Elaphostrongylus cervi* Cameron, 1931, genotype of *Elaphostrongylus* Cameron, 1931, whereas *E. odocoilei* Hobmaier & Hobmaier, 1934, possesses them must be regarded as a possibly significant difference between the two species; but it is suggested that *E. cervi* may be found to have these structures. Therefore *E. odocoilei* is left in *Elaphostrongylus*.

I wish to express my appreciation to Dr Gerard Dikmans, Zoological Division, United States Bureau of Animal Industry, Beltsville, Maryland, and to Dr Frans C. Goble and Mr E. L. Cheatum, Game Research Center, Delmar, New York, for generous assistance during the course of this work.

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A FURTHER NOTE ON THE VIRUSES AFFECTING *ATROPA BELLADONNA* AND A DESCRIPTION OF A VIRUS COMPLEX ATTACKING *HYOSCYAMUS NIGER*

By KENNETH M. SMITH, *Plant Virus Research Station and Molteno Institute, Cambridge*

(With Plate III)

In a previous communication an account was given of an undescribed virus affecting *Atropa belladonna* (Smith, 1943). Further investigation of this plant, which is widely grown on herb farms in the British Isles, has shown that it can apparently act as a symptomless carrier of another virus, first described by Hamilton (1932) and called Hyo III Virus.

The original four plants of *A. belladonna* from which this virus was isolated had been grown out-of-doors and showed no apparent symptoms although selected by the grower as being not entirely normal. Inoculation from these four plants to a series of test plants revealed the presence in each of a virus later identified as Hyo III Virus (*Hyoscyamus Virus I*, Smith, 1937), although the symptomatology on various plants did not seem to correspond entirely with the original descriptions. It is possible that the present virus is a more virulent strain, particularly as it was fatal to all susceptible plants inoculated especially during the winter.

Some experiments were carried out on *A. belladonna* under glass to investigate further its reactions with this virus. A number of seedlings were inoculated with the virus and a similar number colonized with infective aphides, *Myzus persicae*. The aphides had been previously fed for ten minutes on White Burley tobacco plants infected with the virus. No reaction was apparent on any of the *belladonna* seedlings which were kept under observation for four weeks.

After the lapse of another two weeks inoculation from some of these plants back to tobacco showed that two of them were carrying the virus. Virus-free aphides colonized on the remainder picked up the virus from two more plants. It seems clear therefore that *A. belladonna* is a symptomless carrier of *Hyoscyamus Virus I*, although the virus does not appear to enter the plant easily under the experimental conditions since the number of seedlings infected was small.

In testing the reaction of various plants to the virus it was found that certain species of *Nicotiana* responded with the production of very clear, discrete local lesions. This was particularly true of *N. sylvestris* (Pl. III, fig. 1) and of *N. langsdorffii* and *N. rustica*. As a rule the local lesions were followed by a systemic necrosis and death of the entire plant.

The reaction of *Nicandra physaloides* appears to be wholly necrotic and no mottling has been observed. All the middle and lower leaves of the plant are killed by very large necrotic patches. These leaves drop off, leaving a small tuft of leaves at the top of the stem.

All attempts to infect *Datura Stramonium* with the virus were negative.

The tomato plant was found to be susceptible to infection and the effect produced is in the nature of an extremely severe streak disease. Small black lesions usually develop on the inoculated leaves and these are followed after an interval of about a week by a mosaic mottle on the younger leaves. After a further delay the streak symptoms begin to appear; necrotic spots and patches develop on the leaves, while the petioles become entirely necrosed. The leaves finally shrivel completely and hang down, but remain attached to the plant. Necrotic streaks or stripes develop at the same time as the leaf necrosis and run vertically along the stem (Pl. III, fig. 2). Under winter time conditions in the glasshouse the disease was always fatal to the tomato, but it appeared to be less severe under the light and temperature conditions of summer. Severe necroses which may be ring-like in form have been observed on the green fruits of affected tomato plants in the summer. Seed from such fruits gave rise to healthy seedlings.

A DESCRIPTION OF A VIRUS COMPLEX ATTACKING *HYOSCYAMUS NIGER*

Among the affected *belladonna* plants received from the grower were one or two plants of henbane (*Hyoscyamus niger*) which were attacked by a very severe disease. The lower leaves were mostly killed by necrosis, while the tops of the plants were still

green, but much distorted by small necrotic spots. Inoculation from these plants to *Hyoscyamus* seedlings resulted in the death of the latter in every case. Lesions of a ringspot type on the leaves of the inoculated seedlings suggested that potato virus *X* (*Solanum Virus I*) was probably present. This was confirmed by inoculation to White Burley tobacco and *Datura Stramonium*, and by grafting to potato var. King Edward. However, it was clear that a second virus was also present, since inoculation from the affected henbane plants to tobacco produced on the inoculated leaves numerous solid necrotic lesions of a reddish-brown colour in addition to the lesions typical of potato virus *X* (Pl. III, fig. 3). Moreover, if the virus complex was passed through *Datura Stramonium* and back to tobacco, the virus producing the red lesions was eliminated and the *X* virus alone remained.

Attempts were then made to eliminate virus *X* by passing the virus complex through different host plants. During these experiments it was observed that potato virus *X* could easily be transmitted to the cotyledons of ridge cucumber plants on which it produced small necrotic lesions though no systemic infection followed. It was also found that the second virus gave rise to extremely clear and discrete lesions on the inoculated leaves of *Nicotiana sylvestris*. Since passage through various solanaceous plants failed to separate the complex, other non-solanaceous plants were tested, among which was the common turnip. After about a fortnight the turnip plant was observed to be infected with a pronounced vein-banding mosaic. Inoculation from this back to White Burley tobacco produced the reddish lesions previously mentioned, but without the lesions of potato virus *X* which does not infect the turnip plant. Inoculation was next made from this tobacco plant to stock (*Matthiola*) which eventually produced flowers with a colour 'break' (Pl. III, fig. 4).

These facts, together with subsequent tests, confirmed an earlier suspicion that the second component of the *Hyoscyamus* virus complex was *Brassica Virus I*, a common virus affecting cruciferous plants (Smith, 1935) but not previously recorded affecting *Hyoscyamus*.

It is an interesting question as to how the *Hyoscyamus* plants became infected with potato virus *X* which is apparently not insect-borne. Enquiry from the grower showed that the plants had never been in contact with potatoes nor were there any potatoes growing in the vicinity.

SUMMARY

It has been shown that *Atropa belladonna* acts as a symptomless carrier of *Hyoscyamus Virus I*. Some symptoms caused by this virus on other solanaceous plants are described.

A naturally occurring virus complex in *Hyoscyamus niger* was found to consist of *Solanum Virus I* (potato virus *X*) and *Brassica Virus I*. During the course of the investigation it was found that potato virus *X* forms local lesions on the cotyledons of ridge cucumber without systemic infection and that *Brassica Virus I* behaves similarly on the inoculated leaves of *Nicotiana sylvestris*.

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EXPLANATION OF PLATE III

Fig. 1. Inoculated leaf of *Nicotiana sylvestris* showing the local lesions produced by *Hyoscyamus Virus I*.

Fig. 2. Tomato plant infected with *Hyoscyamus Virus I*: note the necrotic stripes on the stem and the severe necrosis and blackening of the leaves.

Fig. 3. Leaf of White Burley tobacco plant inoculated with the virus complex from *Hyoscyamus niger*: note the dark necrotic lesions due to *Brassica Virus I* with the fainter lesions in the background due to *Solanum Virus I* (potato virus *X*).

Fig. 4. Flower of stock (*Matthiola*) inoculated with the virus complex from *Hyoscyamus niger* after previous passage through turnip and tobacco: note the white 'break' in the red flower, characteristic of infection with *Brassica Virus I*.

(Photographs by Roy Markham.)

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STUDIES ON THE NATURE AND PROPERTIES OF THE PERIENTERIC FLUID OF *ASCARIS LUMBRICOIDES*

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(With 13 Figures in the Text)

INTRODUCTION

The body fluid of the nematode is its only internal extracellular fluid and it probably assumes, as far as is necessary, the combined functions of blood, lymph and intercellular fluids of vertebrates. In the case of *Ascaris*, the toxic (reviewed by Koidzumi, 1935), enzymatic (Nakagawa, 1930; Harnisch, 1937), osmotic (Schopfer, 1932) and metabolic (Harnisch, 1935) functions have been examined. Some aspects of this work and the relationship of the body fluid to various metabolic activities have been examined further.

The composition of the body fluid

As a preliminary to studies on the metabolic functions, the composition of the body fluid taken from fresh worms and those which had been subjected to various treatments after removal from the host was determined.

MATERIAL

The materials analysed may be grouped as follows:

(*a*) Body fluid from *A. lumbricoides* of the pig immediately after removal from the host. In this case, samples of whole fluid, or fluid after the precipitation of protein, were kept frozen for 4 hr. until analysis was commenced at the laboratory.

(*b*) Body fluid of parasites 20–24 hr. after their removal from the host. The parasites were transported to the laboratory in thermos flasks containing media similar to that described by Baldwin (1943). (In some instances, as indicated below, phosphate was omitted from the media.) Except when otherwise stated, the temperature of the worms did not fall below 34° C. during transit (a period of about 5 hr.). After arrival at the laboratory the parasites were kept at 37° C. Samples of body fluid were then taken about 20 hr. after the parasites had been removed from the host. As this was the time when fluid for other experiments was obtained, more detailed analyses were carried out with such samples.

(*c*) As for (*b*), but samples were taken at intervals as the period of *in vitro* life increased up to 7 days.

Before obtaining body fluid worms were rinsed in glass-distilled water and dried on filter paper. Fluid was then allowed to drain from the posterior ends of the parasites through fine glass cannulae or small incisions. The crushing of muscular or reproductive tissue was avoided, otherwise the copper-reducing activity of the fluid was very greatly increased. The contamination of the body fluid with exudate from the anus was also avoided.

METHODS

Protein nitrogen was determined by the micro-Kjeldahl method. Reducing sugar (Somogyi's (1937) modification of the Schaffer-Hartmann technique), non-protein nitrogen (micro-Kjeldahl) and chloride (iodometric titration after treatment with silver iodate (Sendroy, 1937, 1937 *a*)) were estimated after the precipitation of protein with sodium tungstate (Van Slyke & Hawkins, 1928). For the determination of inorganic substances a suitable ash was obtained by repeated treatment with fuming nitric acid and perhydrol and taken up in dilute hydrochloric acid. When sodium and phosphorus alone were being determined, ashing was carried out with perchloric and nitric acids. Figures for ash were obtained by heating samples of wet-ashed material with sulphuric acid at 500° C. (The amounts of potassium found in the wet ash were slightly greater than that found in the sulphated ash.) Other determinations were made as follows: sodium—precipitated as the triple acetate and titrated with sodium hydroxide (Weinbach, 1935; Clark, Levitan, Gleason & Greenberg, 1942; Sobel, Kraus & Kramer, 1941); potassium—precipitated as the cobaltinitrite complex (Dreguss, 1931) and estimated by iodometric titration; zinc—colorimetrically as the dithizone compound (Keilin & Mann, 1940); total phosphorus—colorimetrically after the extraction of the molybdenum complex with butyl alcohol (Berenblum & Chain, 1938); iron—colorimetrically as the red ferrous dipyridyl (Hill, 1930); magnesium—precipitated as the 8-hydroxy-quinolinate and titrated iodometrically after bromination with potassium bromate (Greenberg & Mackey, 1932; Greenberg, Anderson &

Tufts, 1935); copper—colorimetrically by the micro method of Eden & Green (1940); haematin—spectroscopically as the pyridine haemochromogen (Elliot & Keilin, 1934); ascorbid acid—by the method of Richter and Croft, 1943. The total solids of the body fluid was found by drying to constant weight at 100° C.

RESULTS

The results concerning those substances occurring in perienteric fluid which were examined further in later experiments are given in detail. In such instances the estimations were carried out in duplicate and, as a rule, the variation and absolute accuracy was within 3 %. Other determinations, the results of which are given in summarized form (see § (*b*)) were usually accurate to within 5 %.

The concentrations of substances found in the body fluid are given in m.mol. per litre. Since the fluid became diluted when worms were kept in media, a correction, based on the average total solid content of the fresh fluid (6·65 g. per 100 ml.) has been applied in parts of §§ (*b*) and (*c*).

(*a*) *The composition of fresh body fluid*

The figures obtained, which are summarized in Table 1, probably show the nature of the fluid when the parasites were in the host. Each analysis was carried out on the pooled fluid from about five large female worms.

Table 1. *Showing the results obtained in the analysis of fresh body fluid.*

Except in the case of total solids, given in g. per 100 ml. body fluid, results are given as concentrations in $M \times 10^{-3}$.

Sample	Glucose	Protein N	Non-protein N	Sodium	Chloride	Total phosphorus	Total solids
1	1·17	497	33·7	128	52·6	12·3	6·61
2	1·38	475	35·2	118	54·9	12·2	6·78
3	1·20	467	38·2	117	59·5	12·3	6·78
4	1·12	525	36·0	119	55·1	11·2	6·71
5	1·25	509	35·5	114	55·6	11·9	6·31

(*b*) *The composition of the body fluid of parasites 20 hr. after their removal from the host*

Results concerning those constituents of the fluid which were examined further (see § (*c*)) are given in detail in Table 2. Table 3 summarizes other results. Fluid from some fifteen large female worms of the same batch was pooled for analysis. Figures given in Table 2 have been corrected for the dilution of the body fluid by means of the factor, average total solids in fresh fluid (6·65 g. per 100 ml.)/total solids found. No close relationship between iron and haematin in the perienteric fluid of five single parasites was found.

The alkaline-copper reducing substances of body fluid were examined further. Though glucose added to the tungstate protein-free filtrate was fermented (using a form of the method of Somogyi, 1927), the copper-reducing power of the normal filtrate was only lowered some 40–60 % by such treatment. The proportion of fermentable sugar was greatly increased, though the amount of other copper-reducing substances was only slightly increased, when the body fluid was contaminated by juices from muscular and reproductive tissues. Such body fluid, which had copper-reducing values equivalent to concentration of $2{\cdot}2\text{–}3{\cdot}5 \times 10^{-3}\,M$ glucose, contained glycogen, giving a red colour with iodine. The presence of glucose in normal body fluid was confirmed by the formation of glucosazone by treatment of a dialysate concentrated by distillation at 50° C. at reduced pressure. The osazone, recrystallized from alcohol, gave a melting-point of 206° C. Though the ferricyanide reducing value (Hagedorn & Jensen, 1923) of the tungstate filtrate was similar to that of the copper-reducing activity, the cadmium sulphate filtrate gave values lower by 10–16 % when estimated by either the Somogyi or Hagedorn-Jensen method. The nitroprusside test for glutathione was negative. Total carbohydrate, determined by the orcinol reaction Tillmans-Philippi (Pirie, 1936) using the tungstate filtrate, reached a concentration equivalent to more than $2 \times 10^{-2}\,M$ glucose for whole body fluid. Uronic acid, estimated by the Tollens naphthoresorcinol reaction (Mozołowski, 1940), was present in the filtrate in small amounts (concentrations less than $2 \times 10^{-4}\,M$ as glucuronic acid). After hydrolysis in 2 *N* hydrochloric acid the estimation was difficult owing to the presence of interfering substances (Hanson, Mills & Williams, 1944). The protein-free filtrate, examined by the method of Elson & Morgan (1933), gave a weak reaction, present after the removal of carbohydrate insoluble in 80 % alcohol, for amino sugar (less than $2 \times 10^{-4}\,M$ as glucosamine). A similar reaction was obtained using the dialysate from whole body fluid. After hydrolysis (Palmer, Smyth & Meyer, 1937), very variable amounts of amino sugar, in concentrations up to $1{\cdot}1 \times 10^{-2}\,M$, were found. Body fluid from male worms, usually, though not always, contained less amino sugar.

(c) *The changes in the composition of the body fluid of starving* Ascaris *ageing* in vitro

The protein nitrogen, non-protein nitrogen, copper-reducing power, sodium, total phosphorus, total solids and chloride of perienteric fluid were determined at intervals as the parasites aged in media similar to that described by Baldwin (1943) with and without phosphate. Each analysis was carried out using the pooled fluid from about five worms taken from the one batch of parasites, any sluggish, white, opaque worms being rejected. If the temperature of the worms fell below 34° C. during transit, they were considered unsuitable for these experiments. The media were renewed at 12 hr. intervals.

Typical results are shown in Figs. 1–13. The averages of the results obtained in the analysis of fluid from worms fresh from the host intestine have been used to give the zero points shown in the figures. Results corrected for dilution (see Fig. 1) are also graphed.

Table 2. *Showing the detailed results of the analysis of body fluid from parasites after* 20 *to* 24 *hr. of* in vitro *life.*

Results (except for total solids) are given as concentrations in $M \times 10^{-3}$ and corrected for the dilution of the body fluid.

Sample	Glucose	Protein N	Non-protein N	Sodium	Chloride	Total phosphorus	Total solids	Arrival temp., °C.
1	1·85	405	30·8	129	56·1	14·5	6·23	29
2	1·42	405	25·2	105	55·9	17·0	6·50	34
3	1·58	435	25·1	135	60·9	17·7	6·29	34
4	1·98	396	25·3	131	56·3	16·5	6·56	34
5	1·52	406	37·9	127	73·0	14·0	5·85	30
6	1·44	435	30·3	121	69·1	13·4	6·42	34
7	1·57	438	33·1	130	71·9	8·2	6·30	34
8	1·19	360	25·1	96	46·1	7·9	6·60	27
9	1·39	433	31·0	143	48·0	9·7	5·52	27
10	1·31	431	30·0	117	54·8	13·0	5·57	27

Table 3. *Showing the summarized results of the analysis of body fluid from parasites after* 20–24 *hr. of* in vitro *life.*

The figures cover the results obtained from ten samples of body fluid except in the case of ascorbic acid and haematin where only five lots of fluid were examined. Figures for ash are given in g. per 100 ml. body fluid, other substances are recorded as $M \times 10^{-3}$ concentrations.

Substance	Average	Limits of variation	Substance	Average	Limits of variation
Copper	< 0·02	—	Haematin	0·028	0·009 to 0·036
Zinc	0·14	0·13 to 0·19	Ascorbic acid	0·05	0·04 to 0·07
Potassium	23·5	18·5 to 32·4	Ash (sulphate)	0·96	0·71 to 1·07
Magnesium	4·95	4·1 to 6·2	Specific gravity	1·008	1·003 to 1·019
Iron	0·13	0·08 to 0·21			

DISCUSSION

(a) *The composition of fresh body fluid*

Compared with the individual variations in the composition of blood of most other species of animals, that of fresh *Ascaris*, as shown in Table 1, is small. The consistency of these results may be partly explained as follows: (1) In these experiments, host animals were in the post-absorptive state when killed, and hence the nature of the intestinal juices would not vary greatly from animal to animal. Since the composition of the host intestinal juices may possibly affect the parasite perienteric fluid by the passage of substances through the cuticle and certainly (Rogers, unpublished) by absorption via the parasite alimentary canal, lack of variation in the fluids surrounding the parasites would lessen variation in the perienteric fluid. (2) The figures given in Table 1 each show the average composition of fluids from five worms.

In composition *A. lumbricoides* body fluid occupies a variable position in relation to blood and tissue juices of other animals. Thus the protein concentration is similar to that in most mammalian lymphs (M'Kendrick, 1889) higher than in intestinal juices (Veirordt, 1893; Pregl, 1895; de Beer, Johnston & Wilson, 1935) and lower than in blood (Abderhalden, 1908; Hayden et al., cited by Dukes, 1937). Non-protein nitrogen is somewhat higher in the parasites and sugar lower. Potassium and magnesium levels are higher than in mammalian blood and slightly lower than in that of terrestrial arthropods (for references on the composition of arthropod blood, see Maluf, 1939).

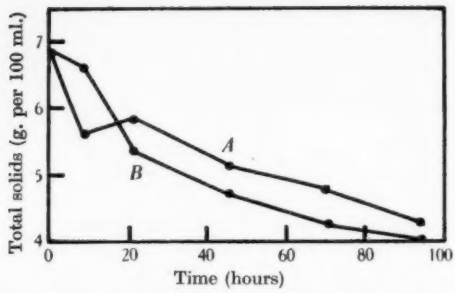

Fig. 1. Showing changes in the total solids of perienteric fluid of starving worms ageing *in vitro*. Curve *A* shows results from parasites kept in media containing phosphate; *B*, worms in media lacking phosphate.

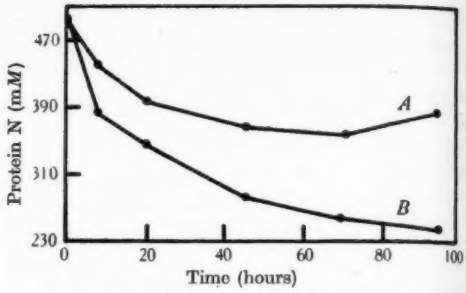

Fig. 2. Showing the changes in the protein nitrogen of the body fluid of starving parasites ageing in media containing phosphate. In curve *A* the results have been corrected for the dilution of the body fluid.

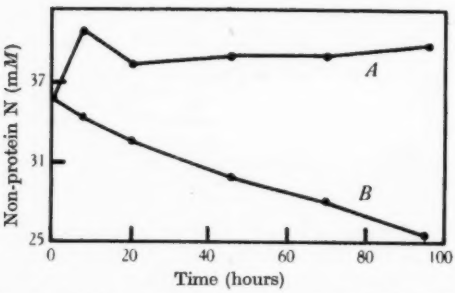

Fig. 3. Showing the changes in the non-protein nitrogen of the body fluid of starving parasites ageing in media containing phosphate. In curve *A* the results have been corrected for the dilution of the body fluid.

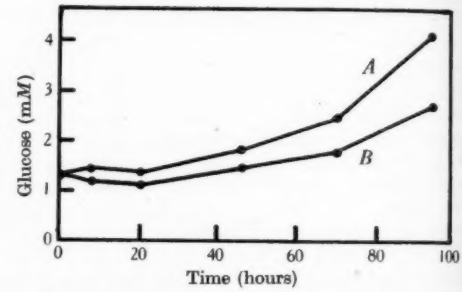

Fig. 4. Showing changes in the copper-reducing power, estimated in terms of glucose, of body fluid from starving parasites ageing in media containing phosphate. In curve *A* the results have been corrected for the dilution of the body fluid.

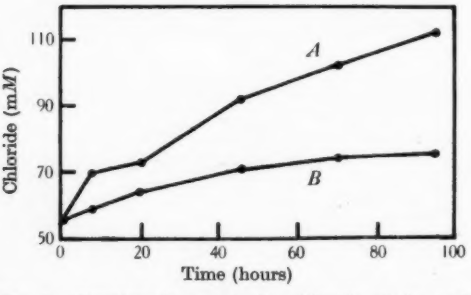

Fig. 5. Showing changes in the chloride of the body fluid from starving parasites ageing in media containing phosphate. In curve *A* the results have been corrected for the dilution of the body fluid.

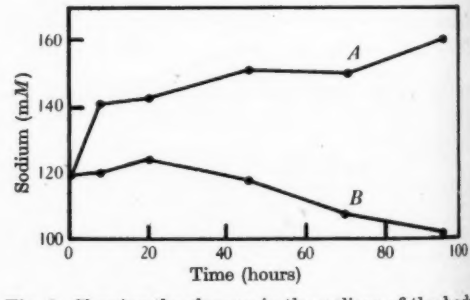

Fig. 6. Showing the changes in the sodium of the body fluid of starving parasites ageing in media containing phosphate. In curve *A* the results have been corrected for the dilution of the body fluid.

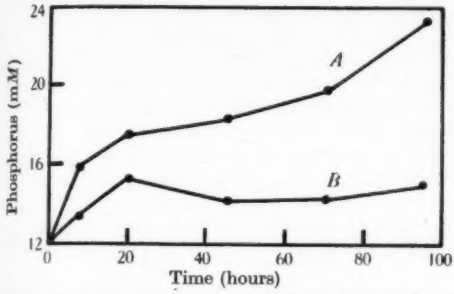

Fig. 7. Showing the changes in the total phosphorus of the body fluid from starving parasites ageing in media containing phosphate. In curve *A* the results have been corrected for the dilution of the body fluid.

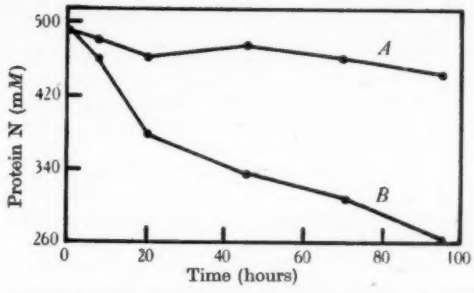

Fig. 8. Showing the changes in the protein nitrogen of body fluid from starving parasites in media lacking phosphate. In curve *A* the results have been corrected for the dilution of the body fluid.

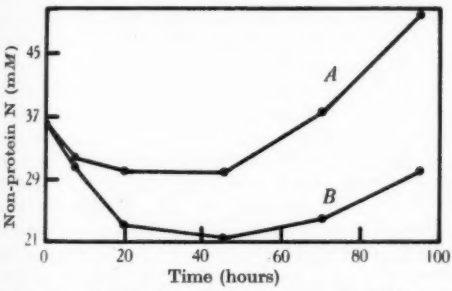

Fig. 9. Showing the changes in the non-protein nitrogen of body fluid from starving parasites ageing in media lacking phosphate. In curve *A* the results have been corrected for the dilution of the body fluid.

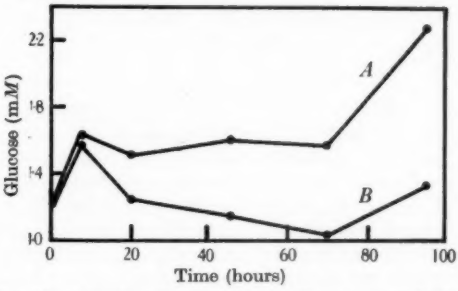

Fig. 10. Showing the changes in the copper-reducing power, estimated in terms of glucose, of body fluid of starving parasites ageing in media lacking phosphate. In curve *A* the results have been corrected for the dilution of the body fluid.

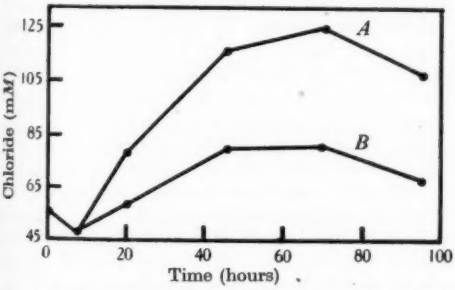

Fig. 11. Showing the changes in the chloride of body fluid from starving parasites ageing in media lacking phosphate. In curve *A* the results have been corrected for the dilution of the body fluid.

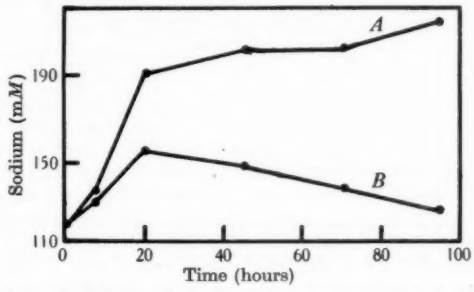

Fig. 12. Showing the changes in the sodium of body fluid from starving parasites ageing in media lacking phosphate. In curve *A* the results have been corrected for the dilution of the body fluid.

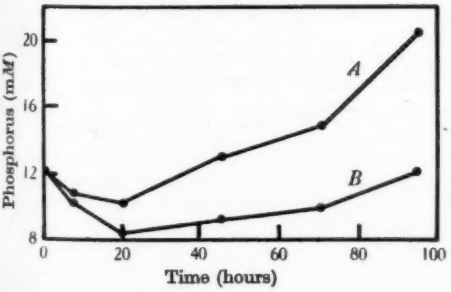

Fig. 13. Showing the changes in the total phosphorus of body fluid from starving parasites ageing in media lacking phosphate. In curve *A* the results have been corrected for the dilution of the body fluid.

Sodium and chloride, though higher in most marine invertebrate fluids (Macallum, 1903; Koizumi, 1935; Bethe & Berger, 1931; Krogh, 1939), mammalian blood, lymph, and cerebrospinal fluid, are lower in terrestrial arthropods.

The body fluid of *A. lumbricoides* differs from most tissues in that the anion concentration, as chloride, is much lower than the cation concentration. This discrepancy is probably partly due to the presence of fatty acids. Though lower fatty acids are probably important metabolites in *Ascaris* (Weinland, 1901, 1904; Schulte, 1917; Flury, 1912, 1913; von Brand, 1934, 1938), their production by worms under various conditions has already been followed by many workers (von Brand, 1934; Toryu, 1936; Oesterlin, 1937; Krüger, 1936, 1937) and has been neglected here.

(*b*) *The composition of body fluid of parasites 20–24 hr. after removal from the host*

Though results were determined by the analysis of mixed fluids from at least fifteen worms, variations between batches of worms were considerable. This was probably largely due to frequent delays during the transit of the parasites to the laboratory during this part of the experiment. It is suggested that changes in body fluid, similar to those found in § (*c*), were greatly accelerated when cooling, due to the delay, occurred.

Though very large amounts of ascorbic acid have been found in the tissues of *Belascaris marginata* (Hill & Smyth, 1944) under the conditions of the present experiments, the amounts found in the body fluid of *Ascaris lumbricoides* are similar to those found in normal blood. The adsorption of the vitamin by intestinal cells of *Ascaris* has been reported (see Hirsch, 1939). The zinc and copper content of perienteric fluid is lower than that of mammalian blood (Burstein, 1929; Elvehjem, Steenbock & Hart, 1929).

Though the total carbohydrate of the body fluid was high, only small amounts of glucose were present, in accordance with the results of other workers (Fauré-Fremiet, 1913; von Brand, 1934). Since some of the amino sugar was dialysable and soluble in 80 % alcohol, it may have contributed, together with the uronic acid, to the reducing activity. The eggs of *Ascaris* have chitinoid shells containing glucosamine (Fauré-Fremiet, 1913) and the presence of amino sugar in the female worm would be expected. Though chitin may form part of the lining of the alimentary tract, the cuticle of *Ascaris* is protein in nature (Flury, 1912; Mueller, 1927, 1929) and contains only a little glucoprotein (Chitwood, 1936). The wide variations in the amounts of amino sugar recorded suggest its presence may have been due to contamination from damaged reproductive tissue. (Much more of such tissue was damaged in obtaining samples from the small male worms.) The contaminant from muscular tissue affecting the reducing power of the body fluid may well have been glycogen, as diastatic enzymes are present (Flury, 1912).

(*c*) *The changes in the composition of the body fluid of* Ascaris *ageing* in vitro

The figures show changes in parasites living under abnormal nutritive, oxidative and osmotic conditions. The latter case is included, for, though the parasite must be subjected to violent variations in osmotic conditions *in vivo* (Krogh, 1939, p. 43) fluctuations from the normal would be of comparatively short duration and mucus surrounding the worms would afford some protection. The changes in the perienteric fluid recorded probably reflect what was largely pathological degeneration in the whole worm.

With respect to time, these changes may be divided into three phases: (I) an early period (0–24 hr.) of fluctuation, (II) an intermediate period of several days when steady changes occurred, and (III) after 5 or 6 (media lacking phosphate) or 7 (media containing phosphate) days of *in vitro* life, a period when the total solids increased rapidly. These phases probably arose in response to (period I) general metabolic disturbances devolving upon the sudden change in environment, (period II) an attempt to establish osmotic equilibrium and (period III) the increasing fragility of tissue cells allowing the rapid leakage of metabolites, etc., into the body fluid. Throughout the whole *in vitro* life the effect of starvation, especially in the case of protein, must have been superimposed on this hypothetical picture.

It is clear that a dilution of the body fluid (Fig. 1) took place. Except during the early stages of *in vitro* life, when there was a consistent sudden fall in the total solids of fluid from worms kept in phosphate media, the dilution was greater in the absence of phosphate. After 5 or 6 days (always later in phosphate media) there was a marked rise in total solids. That dilution was largely due to the entry of water was indicated by increases in the length and weight of worms.

The results suggest that there was an increase in the total amount of sodium in the body fluid, almost enough to maintain its early concentration (see Figs. 6, 12). The rise in chloride was usually different from that of sodium indicating the presence of other important anions. The form and relationship of Figs. 5, 6, 11 and 12 suggest that fatty acid (which is excreted and found in the media surrounding the worms) was increasingly replaced by chloride as the worms aged. The changes in

chloride were more marked when the media lacked phosphate.

Protein invariably showed a marked fall in concentration and usually the total amounts present also fell (Figs. 2, 8). This may be attributed partly to starvation. Non-protein nitrogen rose markedly (sometimes to a concentration of $7{\cdot}0 \times 10^{-2}$ *M*) towards the end of the experimental period, probably as the result of leakage from cells and the hydrolysis of protein.

Total phosphorus (Figs. 7, 13) and the reducing power (Figs. 4, 10) rose steadily when the parasites were kept in media containing phosphate, otherwise there was an early tendency to fall. Later, marked rises in reducing power (concentrations up to $1{\cdot}0 \times 10^{-2}$ *M* as glucose) were not accompanied by large changes in total phosphorus. This was similar to the results obtained when tissue was damaged in obtaining body fluid, leading to elevated reducing values. The nature of the phosphorus compounds in body fluid is probably related to the form of carbohydrate and fat metabolism in *Ascaris* and will be dealt with later in that connexion.

Though the results of the present investigation give indications as to the possible osmotic and metabolic functions of perienteric fluid, they do not warrant a detailed discussion and serve only for an introduction to other work.

SUMMARY

1. The composition of the perienteric fluid of *Ascaris lumbricoides* of the pig, immediately after removal from the host and after varying periods of *in vitro* starvation, is recorded.

2. Apart from the more frequently observed constituents of invertebrate tissue fluids, the body fluid of *Ascaris* was found to contain ascorbic acid, amino sugar and small amounts of uronic acid.

3. Large amounts of anion other than chloride, probably fatty acid, must have been present in the body fluid, though chloride was probably the predominant anion in fluids of parasites which had passed several days of *in vitro* life.

4. The chief factors (starvation, osmotic pressure and the nature of the medium, etc.) affecting the composition of the body fluid are briefly discussed.

The author is greatly indebted to Prof. D. Keilin, Dr T. Mann and Dr A. R. Trim for their interest and advice; and also to Dr S. Elsdon, Dr E. Hartree and Dr D. Bell for advice on analytical methods.

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INDEX OF AUTHORS

BAYLISS, H. A. *'Capsularia marina'* and the Ascaridae of marine hosts, 119

BAYLISS, H. A. Observations on the nematode *Mermis nigrescens* and related species. (With 17 Figures in the Text), 122

CRAGG, J. B. and RAMAGE, G. R. Chemotropic studies on the blowflies *Lucilia sericata* (Mg.) and *Lucilia caesar* (L.), 168

DOUGHERTY, ELLSWORTH C. The lungworms (Nematoda: Pseudaliidae) of the Odontoceti. Part I. (With 13 Figures in the Text), 80

DOUGHERTY, ELLSWORTH C. The nematode lungworms (suborder Strongylina) of North American deer of the genus *Odocoileus*. (With 11 Figures in the Text), 199

GHOSH, S. M. (*see* ROY, D. N.)

GOODRICH, HELEN PIXELL. Coccidian oocysts. (With 6 Figures in the Text), 72

HOLMES, BARBARA (*see* LEA, DOUGLAS)

KEILIN, D. Respiratory systems and respiratory adaptations in larvae and pupae of Diptera. (With Plates I and II and 54 Figures in the Text), 1

LEA, DOUGLAS, SMITH, KENNETH M., HOLMES, BARBARA and MARKHAM, ROY. Direct and indirect actions of radiation on viruses and enzymes. (With 4 Figures in the Text), 110

LUNN, H. F. Observations on the *Sarcoptes* of man. (With 2 Figures in the Text), 67

MARKHAM, ROY (*see* LEA, DOUGLAS)

MILNE, A. The ecology of the sheep tick, *Ixodes ricinus* L. The seasonal activity in Britain with particular reference to Northern England. (With 3 Figures in the Text), 142

MILNE, A. The ecology of the sheep tick, *Ixodes ricinus* L. Host availability and seasonal activity. (With 1 Figure in the Text), 153

RAMAGE, G. R. (*see* CRAGG, J. B.)

REES, GWENDOLEN. A record of parasitic worms from fishes in rock pools at Aberystwyth, 165

ROBINSON, G. G. More cases of abnormal development in the Argasid tick, *Ornithodorus moubata* Murray. (With 2 Figures in the Text), 95

ROGERS, W. P. Studies on the anthelmintic activity of hexylresorcinol and tetrachlorethylene. (With 9 Figures in the Text), 98

ROGERS, W. P. Studies on the nature and properties of the perienteric fluid of *Ascaris lumbricoides*. (With 13 Figures in the Text), 211

ROY, D. N. and GHOSH, S. M. Studies on the population of head-lice, *Pediculus humanus* var. *capitis* De G., 69

SMITH, KENNETH M. A further note on the viruses affecting *Atropa belladonna* and a description of a virus complex attacking *Hyoscyamus niger*. (With Plate III), 209

SMITH, KENNETH M. (*see* LEA, DOUGLAS)

SPROSTON, NORA G. The genus *Kuhnia* n.g. (Trematoda: Monogenea). An examination of the value of some specific characters, including factors of relative growth. (With 7 Figures in the Text), 176

SPROSTON, NORA G. A note on the comparative anatomy of the clamps in the superfamily Diclidophoroidea (Trematoda: Monogenea). (With 1 Figure in the Text), 191

STEPHENSON, WILLIAM. The effects of acids on a soil nematode. (With 3 Figures in the Text), 158

TURK, FRANK A. Studies of Acari. Second series: descriptions of new species and notes on established forms of parasitic mites. (With 19 Figures in the Text), 133

WATSON, J. M. A new sporozoon, *Gregarina rhyparobiae* n.sp., from a tropical cockroach, *Rhyparobia maderae*. (With 16 Figures in the Text), 195

INDEX OF SUBJECTS

Abnormal development in the Argasid tick, *Ornithodorus moubata* Murray, more cases of, 95
Acari, studies of; second series, descriptions of new species and notes on established forms of parasitic mites, 133
Acids, effects of, on a soil nematode, 158
Anthelmintic activity of hexylresorcinol and tetrachlorethylene, studies on the, 98
Ascaris lumbricoides, studies on the nature and properties of the perienteric fluid of, 211

'*Capsularia marina*' and the Ascaridae of marine hosts, 119
Chemotropic studies on the blow-flies, *Lucilia sericata* (Mg.) and *Lucilia caesar* (L.), 168
Coccidian oocysts, 72

Diclidophoroidea (Trematoda: Monogenea), a note on the comparative anatomy of the clamps in the superfamily, 191
Diptera, respiratory systems and respiratory adaptations in larvae and pupae of, 1

Ecology of the sheep tick, *Ixodes ricinus* L.; the seasonal activity in Britain with particular reference to Northern England, 142
host availability and seasonal activity, 153

Fishes, a record of parasitic worms from, in rock pools at Aberystwyth, 165

Gregarina rhyparobiae n.sp., a new sporozoon from a tropical cockroach, *Rhyparobia maderae*, 195

Head-lice, *Pediculus humanus* var. *capitis* De G., studies on the population of, 69
Hexylresorcinol and tetrachlorethylene, studies on the anthelmintic activity of, 98

Ixodes ricinus L., ecology of the sheep tick; the seasonal activity in Britain with particular reference to Northern England, 142
Ixodes ricinus, ecology of the sheep tick; host availability and seasonal activity, 153

Kuhnia n.g. (Trematoda: Monogenea), the genus; an examination of the value of some specific characters including factors of relative growth, 176

Lucilia sericata (Mg.) and *Lucilia caesar* (L.), chemotropic studies on the blow-flies, 168
Lungworms, the (Nematoda: Pseudaliidae) of the Odontoceti. Part I, 80

Mermis nigrescens and related species, observations on the nematode, 122

Nematode, effects of acids on a soil, 158
Nematode lungworms (Suborder Strongylina) of North American deer of the genus *Odocoileus*, 199
Nematode *Mermis nigrescens* and related species, observations on the, 122

Ornithodorus moubata Murray, more cases of abnormal development in the Argasid tick, 95

Pediculus humanus var. *capitis* De G., studies on the population of head-lice, 69
Population of headlice, *Pediculus humanus* var. *capitis* De G., studies on the, 69

Radiation on viruses and enzymes, direct and indirect actions of, 110
Respiratory systems and respiratory adaptations in larvae and pupae of Diptera, 1

Sarcoptes of man, observations on the, 67
Soil nematode, effects of acids on a, 158

Viruses and enzymes, direct and indirect actions of radiation on, 110
Viruses affecting *Atropa belladonna* and a description of a virus complex attacking *Hyoscyamus niger*, a further note on the, 209

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